

77

### THE EFFECT OF GAMMA GLOBULIN FOR APOPTOSIS OF THE KAWASAKI DISEASE PATIENT NEUTROPHILS

Jun-ichi Hirao, Osamu Arisaka, Ken-ichi Sugita, Mitsuoki Eguchi Department of Pediatrics, Dokkyo University, School of Medicine, Tochigi, Japan

(Objective) Intravenous gamma globulin (IVGG) treatment is the most important therapy in the Kawasaki disease (KD), however there are many uncertainties about active mechanism of this treatment. This time, we examined active mechanisms of IVGG treatment affecting on apoptosis of neutrophils. (Patients and controls) Fifteen subjects (4 months ~ 4 years old) were selected from the KD patients who had been admitted to Dokkyo University Hospital between June, 2002 and May, 2001. Healthy adults were used as controls. (Materials and methods) (1) Neutrophils were separated from heparinized blood by the specific gravity centrifugation. (2) Venoglobulin-I was used as human IgG for this experiment. (3) Apoptosis was determined with microscopic examination and flow cytometry. DNA quantity of the cells stained with PI alone and PI and annexin V was measured in flow cytometry. (Results) (1) Spontaneous apoptosis on KD patient neutrophils was more delayed than that on controls, as the picking illness day was earlier. (2) When healthy adult neutrophils was cultured in the presence of IgG (1mg/ml), apoptosis was promoted, but this effect was not observed under less than 0.1mg/ml IgG. Therefore, IgG was used at the concentration of 1mg/ml in further experiments. (3) The morphological changes unlike control neutrophils were observed on KD patient neutrophils after the culture in the presence of IgG. (4) Based on apoptosis pattern of neutrophils in the presence of IgG, samples were divided into two groups, promoted example and not. The promotion of apoptosis by the IgG addition in vitro was observed in about half of samples from the patients, in which IVGG gave more remarkable effects clinically. (Conclusions) We considered the possibility in which one part of the clinical effects of IVGG treatment had appeared by promoting the apoptotic effects to neutrophils.

78

### ADRENOMEDULLIN MRNA IS HIGHLY EXPRESSED IN PBMC OF ACUTE KAWASAKI SYNDROME

Ichiro Nomura<sup>1</sup>, Jun Abe<sup>2</sup>, Hirohisa Saito<sup>3</sup>, Seiji Noma<sup>4</sup>, Donald Y.M. Leung<sup>1</sup> Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO, USA<sup>1</sup>, Dept. of Child Ecology, National Children's Medical and Research Center, Tokyo, Japan<sup>2</sup>, Dept. of Allergy and Immunology, National Children's Medical and Research Center, Tokyo, Japan<sup>3</sup>, Hachioji Metropolitan Children's Hospital, Tokyo, Japan<sup>4</sup>

**Introduction:** Dilation of coronary arteries is a common finding in acute phase Kawasaki Syndrome (KS). The cause of this phenomenon remains unknown. Macrophages and lymphocytes are known to infiltrate into the arterial wall of KS patients. These cells are producers of vasodilatory agents such as nitric oxide synthetase (NOS), calcitonin-related gene protein and adrenomedullin (ADM). The purpose of the current study was to examine the expression of three vasodilators in acute and convalescent KS. **Patients and Methods:** Twenty-two KS patients were enrolled in this study. Blood drawing was performed on around 4th disease day for acute KS and around 20th disease day for convalescent KS patients. Five microgram of RNA from PBMC of each sample was processed to make cRNA and Genechip cDNA microarray (Affymetrix) was performed in four patients. Semi-quantitative RT-PCR was also done with another six KS patients also in acute and convalescent phase. Plasma levels of ADM were assayed in twelve KS patients with RIA kit (Peninsula Labs.). All statistics were performed using Students' paired-t test. **Results:** No differences were observed in mRNA expression by microarray analysis of the vasodilatory agents, NOS and calcitonin-like gene protein between acute vs convalescent phases of 4 patients with KS initially studied. In contrast, ADM showed high-level mRNA expression in acute KS which was 7 fold-higher and showed statistically significant difference ( $p < 0.05$ ) from mRNA expression in convalescent KS. In RT-PCR of 6 additional patients, the same results were obtained. All six patients had high expression of ADM mRNA in acute phase and showed statistically significant difference from convalescent phase ( $p < 0.05$ ). Plasma levels of ADM protein were also high in acute phase and normalized in convalescent phase. **Conclusion:** ADM may contribute to the coronary artery dilation found in acute Kawasaki Syndrome.

79

### INCREASED PLASMA ADRENOMEDULLIN LEVELS IN KAWASAKI DISEASE WITH CORONARY ARTERY INVOLVEMENT

Kouichi Nishida<sup>1</sup>, Ken Watanabe<sup>1</sup>, Kenji Kangawa<sup>2</sup>, Etsuko Tsuda<sup>1</sup>, Osamu Yamada<sup>1</sup>, Yoshikazu Hara<sup>4</sup>, Mitsutoshi Nishimura<sup>4</sup>, Mitsufumi Mayumi<sup>5</sup>, Shigeyuki Echigo<sup>1</sup>, Toshio Nishikimi<sup>5</sup> Department of Pediatrics, National Cardiovascular Center, Osaka, Japan<sup>1</sup>, Research Institute, National Cardiovascular Center, Osaka, Japan<sup>2</sup>, Department of Pediatrics, Fukui Medical University, Fukui, Japan<sup>3</sup>, Department of Pediatrics, Obama hospital, Fukui, Japan<sup>4</sup>, Division of Hypertension and Cardiorespiratory Disease, Dokkyo University School of Medicine, Tochigi, Japan<sup>5</sup>

Adrenomedullin (AM) is a potent vasodilating and natriuretic peptide originally isolated from human pheochromocytoma. The main source of circulating adrenomedullin is now thought to be the vasculature. Kawasaki disease (KD) is an acute febrile illness in young children, characterized by systemic vasculitis preferentially affecting coronary arteries. We hypothesized that plasma AM levels are increased reflecting coronary artery vasculitis in KD. To elucidate this hypothesis, we measured plasma AM levels by radioimmunoassay in six patients with Kawasaki disease (5 male, 1 female, 0.4-2.6 years, 1.3±0.8 years) at before and 3days after high dose intravenous immune globulin therapy and at recovery phase (2 weeks later). In all patients, white blood cell count (WBC) and serum C-reactive protein (CRP) levels increased before treatment (WBC 16500±4509/ul, CRP 11.1±4.1). Compared with normal subjects (9.5±0.5 fmol/ml), plasma AM levels were markedly elevated before treatment. Highest levels of each patient were ranged 58.2 to 141.9 fmol/ml (90.5±35.4 fmol/ml). Specifically, plasma AM levels were remarkably higher in 2 patients who had been detected the coronary artery dilatation by echocardiography (125.6 and 14.9 fmol/ml, each). We believe that the rise in plasma AM in KD is due to the cytokine induced increase of AM expression in vasculature, especially in the coronary artery. Marked elevation of plasma AM at acute phase of KD may help to diagnose the coronary artery involvement in KD.

80

### CHANGES IN PLASMA NITRATE LEVELS IN THE ACUTE PHASE OF KAWASAKI DISEASE

Yumiko Ikemoto<sup>1</sup>, Masayuki Teraguchi<sup>1</sup>, Atushi Ono<sup>2</sup>, Minoru Kino<sup>3</sup>, Ken Yoshimura<sup>4</sup>, Shunji Nogi<sup>4</sup> Department of Pediatrics, Kansai Medical University, Osaka, Japan<sup>1</sup>, Saiseikai Izuo Hospital, Osaka, Japan<sup>2</sup>, Nakano Children's Hospital, Osaka, Japan<sup>3</sup>, Kansai Medical University Kohri Hospital, Osaka, Japan<sup>4</sup>

Aim: Plasma nitrate, the stable end product of nitric oxide(NO), has been reported as an indirect measure of the whole body NO production. The purpose of this study is to measure plasma nitrate in the acute phase of Kawasaki disease, and to evaluate NO production in patients with and without coronary artery lesions. **Methods:** Thirty patients aged 3 months to 6 years were enrolled in this study. Blood samples were obtained serially on the 1, 2, 3, 4, and 8th week of illness. Plasma nitrate was measured by high-performance liquid chromatography. Twenty-six patients were treated with aspirin (10-30mg/kg/day) and intravenous immunoglobulin (2g/kg single dose). Four patients received only aspirin. **Results:** We classified the subjects into 3 groups: normal coronary artery (group N, n=15), coronary dilatation and aneurysm (group D, n=9), transient coronary dilatation (group T, n=6). In all groups, plasma nitrate increased significantly from the 1st week to the 2nd week ( $p < 0.05$ ). Peak levels of nitrate (mean±mm, SEM, μmol/L) in each group were as follows: group N=73.0±15.8, group D=52.3±12.9, group T=58.5±4.4, respectively. Plasma nitrate fell from the 3rd week to the 4th and 8th weeks, but still elevated in each group in comparison with age-matched healthy controls (22.1±8.8): group N=50.9±5.3, group D=46.8±9.3, group T=29.0±1.8. There were no correlations between plasma nitrates and C-reactive protein, neutrophil counts and the Harada score, respectively. **Conclusion:** Increased production of NO in the first 2nd to 3rd weeks of the acute phase was observed. It was consistent with the pathological stage of generalized microvasculitis and myocarditis. Plasma nitrates in group D were lower than those in group N through the course of 8 weeks, indicating decreased NO production due to impairment of the endothelial function.

81

### CIRCULATING ENDOTHELIAL CELLS IN KAWASAKI DISEASE

Keigo Nakatani<sup>1</sup>, Seiichiro Takeshita<sup>2</sup>, Hiroshi Tsujimoto<sup>2</sup>, Youichi Kawamura<sup>2</sup>, Tomoaki Tokutomi<sup>2</sup> Department of paediatrics, Japan Self Defense Forces Sapporo General Hospital, Sapporo, Japan<sup>1</sup>, Department of paediatrics, National Defense Medical College, Saitama, Japan<sup>2</sup>

Recently, endothelial cells (EC) have been reported to be present in the circulating blood of several diseases with vascular injury, and the circulating EC (CEC) contain both the EC which become detached from the vascular wall and endothelial progenitor cells (EPC) which derive from the bone marrow. Kawasaki disease (KD) is widely known to be one type of systemic vasculitis in children. In the present study, we measured the number of CEC (mean±SE cells/ml) in 20 KD patients, who were treated with intravenous immune globulin (IVIG), using anti-EC mAb (clone PIH12)-coated magnetic beads. In 19 KD patients without coronary artery lesions (CAL), the number of CEC in the acute (pre-IVIG:15.7±1.8, post-IVIG:19.1±1.9) and subacute (14.5±1.6) phases was found to be significantly higher ( $P < 0.05$ ) than that in the convalescent phase (8.3±0.9) and healthy controls (HC:3.8±0.7). In one KD patient with CAL, the number of CEC was persistently high (36~44/ml) from the acute through the convalescent phase. The identity of the isolated CEC was confirmed by immunostaining methods using different anti-EC mAbs (VE-cadherin and E-selectin). Furthermore, when the CEC were stained with anti-EPC mAb (clone AC133), EPC were detected in 11 of 20 KD patients. The ratio (%) of EPC/CEC was significantly higher ( $P < 0.05$ ) in the post-IVIG (4.3±1.3) and subacute (5.1±1.7) phases than in both the acute (1.1±0.7) and convalescent (1.3±0.9) phases, and also the HC (0.0±0.0). These findings indicate that the increased number of CEC may be a marker which reflects the process of EC injury in KD vasculitis. Although the major origin of CEC is thought to be the shedding of EC due to vascular injury in KD, the CEC also have a small proportion of EPC which may contribute to the vasculogenesis of KD.

82

### EFFECT OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ON LIVER DYSFUNCTION IN THE ACUTE PHASE OF KAWASAKI DISEASE

Koichi Nihei, Chikako Ikeda, Toshihiko Hosono, Tsugutoshi Aoki, Noriaki Shinomiya Second Department of Pediatrics, Toho University, Tokyo, Japan

Kawasaki disease (KD) is an acute type of systemic vasculitis characterized by a remarkable activation of the inflammatory response. Most KD patients were complicated with the liver dysfunction in the acute phase. To investigate the pathogenesis of the liver dysfunction, we measured the serum levels of inflammatory cytokines including interleukin (IL)-6, interferon-γ, tumor necrosis factor-α, transforming growth factor-β, IL-10, or vascular endothelial growth factor (VEGF), which were related with the pathogenesis of the vasculitis, and the serum levels of albumin and C-reactive protein (CRP) as the indicator of the acute inflammatory response in 35 KD patients. The nineteen of 35 KD patients (54.3%) suffered liver dysfunction (AST50 IU). Neither albumin nor CRP were significantly elevated in the serum of patients with liver dysfunction compared with those without liver dysfunction. Of the measured cytokines, only VEGF was significantly elevated in the patients with liver dysfunction compared with patients without liver dysfunction ( $p < 0.05$ ). We presume that this high level of serum VEGF was caused with the thrombocytosis of KD disease. These results suggest that the liver dysfunction in the acute phase of KD was induced via endothelial cells activated by VEGF.