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POSSIBLE ETIOLOGICAL AGENTS INDUCING MACROPHAGE ACTIVATION AND INCREASED VASCULAR PERMEABILITY IN ACUTE KAWASAKI DISEASE

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Kawasaki disease (KD) is believed to have an infective etiology. However, the agent is not determined. Most KD patients receive drug(s) such as antibiotics just after fever onset. Drug-related vasculitis includes rash, edema and eosinophilia. However, little is known about effects of drugs on KD. We studied peripheral blood eosinophilia (>5%) in 131 patients with acute KD. Eosinophilia was documented in 61 patients (47%), mostly in the first or second week of illness, but not in the age-matched controls ($p<0.0001$). Importantly, circulating levels of interleukin-5 was elevated in almost patients studied, suggesting eosinophil activation. Furthermore, KD microvascular lesions had 20 percent of eosinophils in all fatal KD cardiac tissues studied. Then, we studied effects of antibiotics on child mice immunized with Bacillus Calmette-Guerin (BCG), because BCG potently induces cell-mediated immune reactions. Surprisingly, antibiotics caused hypersensitive symptoms in child mice with BCG, but rarely in those without BCG ($p<0.0001$). Subsequently, coronary vascular mononuclear cell infiltrates with eosinophils were also developed in child mice after antibiotic exposure. Monocyte chemoattractant protein-1 was also expressed in the vascular lesions, mostly in macrophages. These findings suggest that antibiotics may cause coronary vasculitis in susceptible mice. BCG may be a susceptible factor for drug-induced hypersensitivity. Epidemiological survey is urgently necessitated to confirm the association of drugs including antibiotics with the development of KD vasculitis.

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CTLA-4 (CD152) EXPRESSION IN T CELLS DURING THE ACUTE STAGE OF KAWASAKI DISEASE

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Although we have already reported that the activation of monocytes/macrophages plays a central role in acute Kawasaki disease (KD), the role of T cells in KD remains under debate. The kinetics of T cell activation in acute KD was investigated with emphasis on cytotoxic T lymphocytes-associated antigen 4 (CTLA-4, CD152). CTLA-4 is a surface molecule of activated T cells with sequence homology to CD28. Both molecules bind to the same ligands, but have antagonistic functions. While CD28 is an important costimulator, CTLA-4 has an essential inhibitory function in maintaining the homeostasis of the immune system. CTLA-4 is a negative regulator of T cell activation. Using flow cytometry, we investigated intracellular expression of CTLA-4 in CD3+ and CD4+T cells of 13 patients with KD and 13 healthy children. The percentages of intracellular CTLA-4 positive CD3+ and CD4+T cells were 6.6±3.7% and 4±3.8% in KD before treatment during the acute stage (Mean Illness Day: 3.9) . These levels were significantly higher than the levels during the convalescent stage (1.8±1.4%, $p<0.01$ and 1.0±0.9%, $p<0.05$) and were significantly higher than the levels in healthy children (2.4±1.0%, $p<0.01$ and 1.0±0.6%, $p<0.05$). The percentages of CTLA-4+CD3+T cells and CD4+T cells were reduced after intravenous gammaglobulin treatment (3.9±4.2% and 3.3±4.2%, respectively). In this study, we found increases in CTLA-4 expression in T cells during acute KD. Since CTLA-4 is an inhibitory molecule of T cell activation, peripheral blood T cell activation seemed to have terminated by the time KD patients were diagnosed. Peripheral blood T cells might activate before symptoms appear in KD patients.

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CD8+ T LYMPHOCYTES AND MACROPHAGES INFILTRATE CORONARY ARTERY ANEURYSMS IN ACUTE KAWASAKI DISEASE

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The pathogenesis of coronary arterial inflammation in acute Kawasaki Disease (KD) is unclear. Immunophenotypic characterization of the inflammatory cells in the KD vascular lesion has been reported previously in only a single case, by Terai and colleagues. To test the hypothesis that the vascular lesion in KD is an activated T lymphocyte-dependent process, we performed immunohistochemical studies on coronary artery aneurysms from eight fatal acute KD cases using antibodies to CD45RO (activated/memory T lymphocyte), CD8 (cytotoxic/suppressor T lymphocyte), CD4 (helper T lymphocyte), HAM56 (macrophage), and CD20 (B lymphocyte). We found that acute KD coronary arteritis was characterized by transmural infiltration of CD45RO+ T lymphocytes, with four- to five-fold more CD8+ T lymphocytes compared with CD4+ T lymphocytes. Macrophages were present primarily in the adventitial layer, and CD20+ B lymphocytes were notably absent. These data lend support to the hypotheses that KD results from infection with an intracellular pathogen such as a virus whose antigens are presented by MHC class I molecules, and that CD8+ T lymphocytes and macrophages are important in the pathogenesis of KD coronary aneurysms.

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EXPRESSION OF APOPTOSIS INHIBITOR GENES AND RESISTANCE OF T CELLS TO APOPTOSIS IN THE EARLY ACUTE PHASE OF KAWASAKI DISEASE

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Objective. The acute phase of Kawasaki disease (KD) is characterized by the deregulated production of proinflammatory cytokines and chemokines by PBMC. We studied the activation induced cell death of the peripheral blood T cells and the expression of the apoptosis-related genes during the course of the disease. **Methods**. Heparinized blood was obtained from 63 patients, 2-4d (Group A, n=25), 5-7d (Group B, n=29), 8-11d (Group C, n=9), 12-14d (Group D, n=9), and >15d (Group E, n=13) after the onset of fever. PBMC were isolated 24h after blood drawing and stained with anti-CD3 antibody and Annexin V followed by the flow cytometry analysis. The expression of the apoptosis-related genes was studied on PBMC obtained during the acute (<6d) and convalescent (>11d) phase of KD by RT-PCR. **Results**. The percentage of apoptotic T cells was not increased in the early acute phase (Group A, 13.3%), but gradually increased during the subacute phase with the peak value in the Group C (26.2%, $p=0.0002$ by Fisher's PLSD). After IVGG therapy, the percentage of apoptotic T cells was significantly increased (15.2% vs. 20.2%, $p=0.02$ by unpaired t test). The expression of the anti-apoptotic protein genes such as FLICE-like inhibitory protein (FLIP), X-linked inhibitor of apoptosis protein (XIAP), and Bcl-XL was significantly elevated during the acute phase compared with the convalescent phase of KD. **Conclusion**. The enhanced expression of the apoptosis inhibitory proteins during the early acute phase of KD may prevent the induction of activation induced cell death of PBMC and contribute to the sustained production of proinflammatory cytokines during the acute illness.

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MODULATION OF ADHESION MOLECULE EXPRESSION BY TNF-ALPHA IN HUMAN CORONARY ARTERY ENDOTHELIAL CELLS: INSIGHTS INTO THE PATHOGENESIS OF KAWASAKI SYNDROME

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Background: Kawasaki syndrome (KS) is characterized by acute elevations of pro-inflammatory cytokines, matrix metalloproteinase 9 (MMP-9) and other factors related to vascular remodeling. The events leading to coronary artery damage in KS are unknown. Methods: Human coronary artery endothelial cells (HCAEC), treated with TNF-α or pre-treated with various concentrations of salicylic acid or TGF-β prior to stimulation with TNF-α were analyzed for gene and protein expression of adhesion molecules by RT-PCR and cell-based ELISA, respectively. Results: TNF-α up-regulated ICAM-1, E-selectin and MCP-1 gene and protein expression in a time- and dose-dependent manner via the NF-κB pathway, which could be inhibited by salicylic acid, but not TGF-β. By contrast, cultured HCAEC did not produce MMP-9 when induced with TNF-α. However, we did observe increase in MMP-9 enzyme levels in peripheral blood mononuclear cells from acute phase of KS patients compared to the convalescent phase when cultured *in vitro*, without any stimulation. Conclusions: The ability of HCAEC to express adhesion molecules and secrete MCP-1 upon exposure to TNF-α allows us to formulate a hypothesis for the pathogenesis of KS: namely, MCP-1 provides a gradient to attract immune cells to sites of inflammation, allowing these cells to attach to the endothelium and undergo extravasation into the extracellular matrix. Once there, the immune cells release enzymes that assists in vascular remodeling, thereby weakening the endothelium and hastening the process of aneurysm formation. In addition to preventing thrombosis and lowering fever in KS, salicylic acid may also function in down-regulating the expression of adhesion molecules during the inflammatory stage. [U.S. Public Health Service grant G12RR/AI-03061 from the RCMI Program, NCRR, NIH]

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CRITERIA OF MYOCARDIAL FRACTIONAL FLOW RESERVE AND CORONARY FLOW RATIO FOR DETECTION OF MYOCARDIAL ISCHEMIA IN PATIENTS WITH KAWASAKI DISEASE

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Purpose of this study is to estimate the criteria of myocardial fractional flow reserve (FFR_{myo}) and coronary flow ratio (CFR) for detection of silent myocardial ischemia. 112 patients (1y-15y) were divided into three groups; normal coronary or coronary dilation without stenosis (N) group (n=61), coronary stenosis without ischemia (n-IS) group (n=30), myocardial ischemia (IS) group (n=21) by 2-D echo, and rest and exercised myocardial scintigraphy. FFR_{myo} is defined as the ratio of maximal achievable flow in myocardium subtended by a stenosed coronary artery to the maximal achievable myocardial flow induced by papaverine. CFR was calculated as a ratio of averaged peak velocity before and after papaverine. FFR_{myo} and CFR were calculated and compared among three groups. Sensitivity and specificity for detection of myocardial ischemia were calculated by abnormal values of FFR_{myo} and CFR. Moreover, FFR_{myo} and CFR tests estimated effectiveness of PTCA and CABG. Results: Criteria for detection of myocardial ischemia were defined the mean±2sds for values of FFR_{myo} and CFR in N group; <0.75 in FFR_{myo} and <2.0 in CFR. FFR_{myo} and CFR in IS group (0.64±0.3#, 1.2±0.4#) were significantly decrease (# $p<0.05$). Sensitivity and specificity for detection of myocardial ischemia were very high by FFR_{myo} and CFR. Moreover, FFR_{myo} and CFR were very useful index for analysis of coronary blood flow velocity and pressure dynamics before and after PTCA and CABG. Conclusions: The values of <0.75 in FFR_{myo} and <2.0 in CFR can be useful to estimate myocardial ischemia and to evaluate the effectiveness of PTCA and CABG.