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ELEVATED LEVELS OF MATRIX METALLOPROTEINASE-9 AND TISSUE INHIBITOR OF METALLOPROTEINASE-1 IN THE ACUTE PHASE OF KAWASAKI SYNDROME

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Background: Kawasaki syndrome (KS) is characterized by inflammation of coronary arteries and other medium-sized muscular arteries, leading to coronary aneurysms and thromboses. Matrix metalloproteinases (MMPs), a class of zinc-dependent, calcium-requiring enzymes capable of degrading extracellular matrices, are vital for extracellular remodeling. Elevated MMP-9 mRNA, protein and enzyme levels have previously been reported in adults with abdominal aortic aneurysm. Methods: Plasma or serum samples from KS patients (31) and febrile (9) and afebrile (17) controls were analyzed for MMP-9 enzyme and protein levels, as well as TIMP-1 protein levels, by gelatin zymography and ELISA. Results: Both MMP-9 enzyme and protein levels and TIMP-1 protein levels were significantly elevated in the acute phase of KS compared to their respective convalescent phase (mean MMP-9 enzyme: 114,754 pixels vs. 29,523 pixels; MMP-9 protein: 1,147 ng/mL vs. 193 ng/mL; TIMP-1 protein: 1,321 ng/mL vs. 651 ng/mL), as well as to febrile (MMP-9 enzyme: 114,754 pixels vs. 54,947 pixels; MMP-9 protein: 1,147 ng/mL vs. 428 ng/mL; TIMP-1 protein: 1,321 ng/mL vs. 600 ng/mL) and afebrile (MMP-9 enzyme: 114,754 pixels vs. 25,071 pixels; MMP-9 protein: 1,147 ng/mL vs. 110 ng/mL; TIMP-1 protein: 1,321 ng/mL vs. 552 ng/mL) controls. Conclusions: The markedly elevated levels of MMP-9 in the acute phase of KS may reflect vascular remodeling or an inflammatory response to a microbial agent. Furthermore, because MMP-9 and TIMP-1 in KS are significantly different from febrile controls, we are evaluating the usefulness of MMP-9 and TIMP-1 as a much needed diagnostic test for KS. [Supported by grants from RCMI program (G12RR/AI-03061), NCRR, NIH; American Heart Association (9960338Z); and Hawaii Community Foundation (990565)]

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THE IMBALANCE BETWEEN MATRIX METALLOPROTEINASE-9, -2 AND TISSUE INHIBITOR OF METALLOPROTEINASES-1 IN ACUTE PHASE KAWASAKI DISEASE

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Background: Matrix metalloproteinases (MMPs) have been considered to play a pivotal role in extracellular matrix breakdown. The activity of MMPs is closely regulated by tissue inhibitors of metalloproteinases (TIMPs) under the pathophysiological conditions. In recent studies, the quantitative imbalance between certain MMPs and TIMPs may play an important role in the vascular remodeling process. Objective and Methods: To investigate the role of MMPs and TIMPs in vasculitis of patients with acute phase Kawasaki disease (KD), we sequentially measured the serum levels of MMP-1, 2, 3, TIMP-1, -2, and the plasma levels of MMP-9 using rapid one-step sandwich enzyme immunoassay before and after IVGG treatment. Nineteen patients (13 boys and 6 girls; 17.4±10.1 months of the mean age) with acute KD were studied. Results: The serum levels of MMP-1 and -3 before treatment were slightly elevated compared to normal range of adults reported in previous literatures and returned to normal range after 1 month. MMP-2 levels were slightly elevated before treatment and remained that level through 1 month later ($p<0.01$). The plasma levels of MMP-9 and serum levels of TIMP-1 were significantly elevated before treatment and returned to normal range 1 month later ($p<0.05$, $p<0.01$). Consequently, the ratio of MMP-2/TIMP-1 decreased, and MMP-9/TIMP-1 increased gradually after the IVGG treatment ($p<0.01$, $p<0.01$). Conclusion: We speculate that the imbalance between MMP-9, MMP-2 and TIMP-1 may play an important role in acute phase KD.

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THE EFFECT OF INTRAVENOUS IMMUNOGLOBULIN ON INHIBITING PERIPHERAL BLOOD LYMPHOCYTE APOPTOSIS IN ACUTE KAWASAKI DISEASE

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*Aim:*To further explore the therapeutic mechanism of intravenous immunoglobulin(IVIG) for Kawasaki disease(KD). *Method:* Peripheral blood lymphocytes(PBLs) obtained from 26 children with KD and 20 age matched healthy children, were stimulated with anti-CD3 monoclonal antibody-(mAb),and apoptotic cell percentage and DNA fragmentation were assayed at 0,12,24,48,72 hours in vitro. The patients belonged to 2 groups: One treated with aspirin combined with IVIG($n=16$) and one treated with aspirin alone($n=10$).PBLs were stimulated by phytohemagglutinin(PHA) to evaluate lymphocyte proliferative response. *Results:* Compared with normal controls, the apoptotic cell percentage and the DNA fragmentation were markedly decreased($p<0.001$) and delayed in PBLs from KD patients. After IVIG treatment, the decreased percentage of apoptotic cell and delayed DNA fragmentation were restored to the state of the normal controls, accompanied by a fast clinical remission as compared to the Asp alone group. The lymphocyte proliferative response was also decreased 3-5 days after IVIG therapy ($p<0.001$). *Conclusion:* The results suggested that decreased PBL apoptosis might be involved in the pathogenesis of KD. The therapeutic mechanism of IVIG in KD may be partially due to the reversal of the inhibited lymphocyte apoptosis, and may have implications for other autoimmune diseases with inefficient lymphocyte apoptosis.

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THE EFFECT OF IL-6 ON PERIPHERAL BLOOD LYMPHOCYTE APOPTOSIS IN ACUTE KAWASAKI DISEASE

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Aim: To further explore the pathogenesis of Kawasaki disease (KD). *Methods:* Observing cell morphological change, Calculating percentage of apoptotic cells and assaying DNA fragmentation in peripheral blood mononuclear cell (PBMC) stimulated by anti-CD3 for 0, 12, 24,48,72h in Vitro from 30 KD patients and 20 age-matched health children. *Results:* Apoptotic cell percentage and DNA fragmentation were markedly decreased ($P<0.001$) and delayed(in comparison with those in normal controls),which could reach the states of control group only by 72h culture. Remarkably increased production of IL-6 in cultured supernatants of PBMC induced by phytohemagglutinin (PHA) from KD patients was found in comparison with those of PBMC from controls ($P<0.001$). Adding anti-IL-6mAb to the cultures or using intravenous immunoglobulin (IVIG) in Vivo led to significantly decreased production of IL-6 ($P<0.001$),and decreased apoptotic cell percentage and delayed DNA fragmentation reversed to be compatible with normal controls. *Conclusion:* Peripheral blood lymphocyte apoptosis is inhibited in KD and over-produced IL-6 might be involved in this process.

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STUDY ON THE MECHANISM OF INHIBITED PERIPHERAL BLOOD LYMPHOCYTE APOPTOSIS IN CHILDREN WITH ACUTE KAWASAKI DISEASE

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Aim: To explore the Mechanism of inhibited peripheral blood lymphocyte apoptosis in children with Kawasaki disease. *Methods:* Calculating percentage of apoptotic cells and assaying DNA fragmentation in peripheral blood lymphocyte(PBL) in vitro from 30 kawasaki disease(KD) patients and 20 age-matched healthy children, measuring in vitro production of IL-6 by ELISA, expression of PBL p53 gene expression using dot-blot and flow cytometry (FCM). *Results:* Apoptotic cell percentage and DNA fragmentation were markedly decreased ($P<0.001$) and delayed,compared with those in normal controls, which could reach the states of the control group only being cultured for 72h, remarkebaly increased production of IL-6 in cultured supernatant of PBL induced by phytohemagglutinin (PHA) from KD patients was found, and the expression of PBL p53 gene expression was decreased in KD patients. Adding anti-IL-6 monoantibody(mAb) to the cultures or using intravenous immunoglobulin (IVIG) in vivo led to significantly decreased production of IL-6($P<0.001$), and reversed decreased apoptotic cell percentage and delayed DNA fragmentation, while the expression of PBL p53 gene increased. *Conclusion:* The data suggested that PBL apoptosis is delayed in KD patients. It might be related to over-production of IL-6 inhibiting expression of p53 gene.

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NEUTROPHIL APOPTOSIS IS INHIBITED IN THE ACUTE PHASE OF KAWASAKI DISEASE

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[Objectives] Circulating polymorphonuclear neutrophils(PMNs) are known to increase in number and are functionally activated in the acute phase of Kawasaki disease(KD). The aim of the present study is to investigate whether the apoptosis of PMNs is deregulated in acute KD. [Patients and methods] We studied the apoptosis of PMNs (AnnexinV-positive cells and cells with fragmented DNA) and the expression of Fas(CD95) and Bcl-2 protein(A1, Bax) using flow cytometer in KD patients($n=25$), patients with a bacterial infection(BI, $n=20$), viral infection(VI, $n=20$) and healthy children(HC, $n=20$). [Results] When the isolated PMNs were cultured in vitro, the proportions of spontaneous apoptotic PMNs were found to be significantly lower ($P<0.01$) in the acute KD patients than in BI, VI or HC. The proportion of circulating Fas(CD95)-positive PMNs was also significantly lower ($P<0.01$) in the acute KD patients than in the other groups. In the acute phase of KD, the proportion of spontaneous apoptotic PMNs showed both a significant negative-correlation ($P<0.01$) with the peripheral PMN counts and a significant positive-correlation ($P<0.01$) with the proportions of circulating Fas(CD95)-positive PMNs. Furthermore, the agonistic anti-Fas mAb(CH-11) induced a significant increase in the proportion of apoptotic PMNs in the patients with a viral infection and healthy children, but not in either the patients with acute KD or the patients with a bacterial infection. In the intracellular expression of anti-(A1) and pro-apoptotic protein(Bax), the A1/Bax ratio was significantly higher in acute KD than in the other groups. [Conclusions] These findings indicate that PMN apoptosis is inhibited during the acute phase of KD and also suggest that both the resistance against the Fas-mediated death signal and the down-regulation of the mitochondrial apoptotic signaling pathway due to an altered balance of Bcl-2 protein expression are responsible for the delayed PMN apoptosis.