

65

CLONING AND DOMAIN EXPRESSIONS OF *STREPTOCOCCUS MITIS*-DERIVED HUMAN PLATELET AGGREGATION FACTOR (SM-HPAF) GENE IN *ESCHERICHIA COLI*

Hisashi Ohkuni¹, Yukino Watanabe², Yuko Todome², Hidemi Takahashi², Kazuhito Ohkura³, Hideaki Nagamune³, Yoshinobu Hishina¹ Clinical Laboratory, Medea Japan Co., Ltd., Saitama, Japan¹, Department of Microbiology and Immunology, Nippon Medical School, Tokyo, Japan², Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, Tokushima, Japan³

We have purified and partially characterized a human platelet aggregation factor (Sm-hPAF) from extracellular products of *Streptococcus mitis*, strain Nm-65 isolated from a patient with Kawasaki disease. Chemical analysis revealed that Sm-hPAF contains first 15 amino-terminal residues were N-DEQGNRPVETENIAR. Based on this partial sequence, *sm-hpaf* that encodes Sm-hPAF was amplified by PCR. Sequence analysis indicated that *sm-hpaf* encodes 665 amino acids with 36 residues as signal sequence. Deduced amino acid sequence of *sm-hpaf* without signal peptides was named Sm-hPAF. It suggested that partial sequence was similar to cytolysin family (Perfringolysin O, Intermedilysin, and Streptolysin O etc.), but no homology/similarity was seen in N-terminal sequence of Sm-hPAF. It was suggested that Sm-hPAF had new domain except for four domains that were highly conserved among cytolysin family and designated as domain 0. Each domain was amplified and cloned into pQE 30, 31 and 32 vectors. The vectors were transformed into *Escherichia coli* M15[pREP4] and expressed with IPTG. Aggregation activity of each recombinant product is in progress.

66

"CYTOVIRIDIN(E)", A NOVEL ETIOLOGIC METABOLITE CHARACTERIZING KAWASAKI DISEASE IS AN ANALOGUE OF HUMAN SERUM ALBUMIN; CYTOTOXIC PROTEINASE BLOCKING CYTO-CHROMS AND AGGREGATING MAMMALIAN THROMBOCYTE

Yasushi Miyamoto¹, Junko Tanaka² Formerly, Dpt. Of Bacteriology & Pathology, Kanagawa Pref. Pbl. Hlth. Lab. Yokohama, Japan¹, Pharmaceutical Research Center, Meiji Seika Kaisha Ltd. Pharmacology Dpt., Drug Research Lab., Yokohama, Japan²

For simplifying analytic process, the preceded few fractions of the chromatographed effluent were put aside and stored for later studies. Their extremely high protein content were connotative of the existence of a polymer deriving most probably from the above albumin-analogue. For convenience sake, we wish to use "albuminoid" and "globulinoid" respectively. The MW of albuminoid was 66.2 kd; whereas that of an albumin is 66.0 kd; almost the same values with a similar pI value. Drop of blood-albumin level of patients might be ascribable to the elaboration of albuminoid by the organism, i.e., viridans group streptococci; at least two species take the role, e.g., *S. sanguis*, *S. parasanguis* or *S. oralis*. They usually cooperate for this *in situ* amino acid and/or peptid synthesis. Thus, the relation between patient (host) and organism (parasite) is competitive for depriving these raw materials. Things might be similar in case of globulinoid synthesis; raw materials must be deprived by the organism: rapid improvement of clinical symptoms brought about after gamma-globulin therapy must be due to its genuine complementary efficacy. General views cited above were based on our data on all the stage of molecular evolution, from a precursor peptide through albuminoid up to globulinoid. Data might be applicable to members of similar genom family.

67

SEROLOGICAL ANALYSIS OF AUTOANTIGENS OF KAWASAKI DISEASE BY CDNA EXPRESSION CLONING

Miho Kaneko¹, Toshiro Ono², Tomoyo Matsubara¹, Eiichi Nakayama², Susumu Furukawa¹ Department of Pediatrics, Yamaguchi University School of Medicine, Yamaguchi, Japan¹, Department of immunology, Okayama University Graduate School, Okayama, Japan²

Kawasaki Disease (KD) is an acute vasculitis of young childhood. However, little is known about the pathogenesis and immune response of KD. In this study, autoantigens of KD recognized by the host humoral immune response were investigated by serological analysis of antigens by recombinant expression cloning (SEREX). Immunoscreeing of a cDNA expression library from human umbilical vascular endothelial cells, which have been activated by IL-1 and TNF- α , with serum from a KD patient identified more than 20 autoantigens, including several antigen processing related proteins. To analyze whether immunorecognition of these autoantigens was KD related, allogeneic sera samples obtained from normal blood donors, patients with KD and patients with other diseases were tested for reactivity against the autoantigens defined in the study.

68

LEUKOCYTE ADHESION FACTOR MAC-1 EXPRESSION ON GRANULOCYTE ASSOCIATES WITH VASCULITIS IN KAWASAKI DISEASE

Ryuji Fukazawa, Yoeko Uchikoba, Yukio Kuramochi, Ei Ikegami, Mitsuhiro Kamisago, Takashi Seki, Shunichi Ogawa Department of Pediatrics, Nippon Medical School, Tokyo, Japan

We have revealed massive expression of Ca binding protein myeloid-related protein (MRP) on circulating leukocyte in acute phase of Kawasaki disease. Newton et al. (J Immunol 1998 160:1427) reported MRP reinforce the ability of adhesion molecule Mac-1, which suggest the keen relationship between MRP, Mac-1 and vasculitis. We quantified leukocytes Mac-1 expression in Kawasaki disease, and evaluated the adhesion ability between cultured human coronary artery endothelial cell and Kawasaki disease patients' peripheral leukocyte. (*Materials and Methods*) mRNA was extracted from the Kawasaki disease patients' leukocyte (n=21) and was converted to cDNA by RT-PCR, and Mac-1 expression was evaluated by quantitative PCR (Applied Biosystems; GeneAmp 5700). The patients' leukocyte, labeled with BCECF-AM, exposed to cultured human coronary artery endothelial cell, and leukocyte adhesion assay was performed. (*Result*) Mac-1 expression was a peak on acute phase of Kawasaki disease and significantly decreased after 1 month of onset. The patients' leukocyte adhesion ability to endothelial cell was significantly increased, which was significantly inhibited by addition of anti-Mac-1 antibody. We postulated Mac-1 play the key role for leukocyte invasion into endothelium, which is the initial step for causing vasculitis.

69

MATRIX METALLOPROTEINASES-2 AND-9 IN AREAS OF EXTRACELLULAR MATRIX DESTRUCTION AND ANGIOGENESIS IN CORONARY ARTERY ANEURYSMS IN KAWASAKI DISEASE

Patrick J. Gavin¹, Susan E. Crawford², Stanford T. Shulman¹, Anne H. Rowley¹ Division of Infectious Diseases, The Children's Memorial Hospital, Chicago, IL, USA.¹, Department of Pathology, Northwestern University Medical School, Chicago, IL, USA.²

Coronary artery aneurysms (CAA) can complicate Kawasaki Disease (KD) and lead to rupture, coronary thrombosis or coronary stenosis. CAA are characterized histologically by destruction of extracellular matrix (ECM), most notably elastic tissue in the vascular media; inflammatory cell infiltrates; and neovascularization. Matrix metalloproteinases (MMP) are known to regulate degradation and remodeling of the ECM and angiogenesis. MMP-2 and -9 have been implicated in the pathogenesis of abdominal aortic aneurysms and atherosclerosis, and elevated plasma levels of MMP-9 have been demonstrated in acute KD. We hypothesized that MMP are important in the pathogenesis of CAA development and vascular remodeling in KD. To determine if MMP are present in KD CAA, we performed immunohistochemistry for MMP-2 and -9 on paraffin-embedded formalin-fixed coronary artery tissue from 11 fatal acute KD cases and from 7 children who died of other causes. Although there was no significant quantitative difference in MMP-2 in KD and non-KD coronary arteries, there was a qualitative difference in the pattern of MMP-2 expression. In control and non-aneurysmal KD arteries, MMP-2 was present only in vascular smooth muscle cells and endothelial cells, whereas in KD CAA, MMP-2 was prominent in the thickened neointima, in smooth muscle cells migrating from the media into the neointima, and in endothelial cells in areas of adventitial angiogenesis. In contrast, there was a significant difference in the expression of MMP-9 in KD CAA and control coronary arteries (p \leq 0.01). MMP-9 was prominently expressed by mononuclear inflammatory cells in CAA but was not expressed in non-aneurysmal KD coronary arteries or in control subject coronary arteries. We conclude that MMP-2 and -9 are differentially expressed in CAA of KD patients when compared to non-aneurysmal KD or control coronary arteries. Differential expression of MMP may contribute to ECM degradation, neointimal proliferation, and local angiogenesis in KD CAA.

70

MATRIX METALLOPROTEINASE-9 (MMP-9) REGULATION BY CYTOKINE NETWORK CENTERING ON HUMAN HEPATOCYTE GROWTH FACTOR (HHGF) IN KAWASAKI DISEASE

Koichi Sakata¹, Sei-ichiro Ozawa¹, Kenji Hamaoka¹, Masato Nishimura², Shoji Ushiyama² Division of Pediatrics, Children's Research Hospital, Kyoto Prefectural University of Medicine, Kyoto, Japan¹, Department of Laboratory Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan²

Background We reported that MMP-9, regulated by various cytokines, could play an important part of vascular remodeling in Kawasaki disease (KD). However, it is still unclear what kind of interaction among cytokines (as cytokine network) regulates MMP-9 expression. Therefore, we focused on hHGF, one of the strongest angiogenic factors produced by various cells including vascular component cells, and investigated the regulatory mechanisms of MMP-9 under cytokine network centering on hHGF in KD. *Subjects* 30 KD patients (group KD: M 19, F11; 3m-5y), 10 healthy controls (H: M 5, F 5; 5m-3y) and 10 febrile controls (F: M5, F5; 10m-4y). *Methods* Plasma MMP-9 and hHGF were measured by ELISA. After treatment with plasma samples or cytokines including hHGF, the levels of mRNA for MMP-9, hHGF and cMET in HUVEC and fibroblasts were detected by RT-PCR (quantitative or not). *Results* Plasma MMP-9 and hHGF levels markedly increased during all phases in KD (MMP-9: H 31.8 \pm 12.1 ng/ml, F 109.9 \pm 87.1, KD pre-IVIG 304.5 \pm 269.0, post-IVIG 130.5 \pm 116.5, 1 m 77.7 \pm 73.4 and hHGF: 0.09 \pm 0.06 ng/ml, 0.32 \pm 0.16, 0.92 \pm 0.49, 0.37 \pm 0.19 and 0.45 \pm 0.69, respectively). There was a significant positive correlation between MMP-9 and hHGF. The assessed levels of mRNA for MMP-9 in HUVEC were significantly higher in KD pre-IVIG phase, and stimulated by hHGF in a dose dependent manner. IL-6 enhanced hHGF expression in fibroblasts. Messenger RNA for cMET was significantly enhanced in HUVEC under co-culture with IL-6 stimulating fibroblasts, and was suppressed by additional using anti-hHGF antibody. *Conclusion* It was suggested that hHGF, produced by fibroblasts stimulated by other cytokines, could regulate the synthesis of MMP-9 by endothelial cells in KD.