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Elastolytic Matrix Metalloproteinases and Coronary Outcome in Children with Kawasaki Disease

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ABSTRACT: Kawasaki disease (KD) is a multisystem vasculitis that leads to coronary artery damage and aneurysm formation. Elastolytic matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of arterial aneurysms. To determine the relationship between circulating levels of elastolytic MMPs and development of coronary artery aneurysms in children with KD, we partnered studies done in affected children with an animal model of disease. In affected children, circulating protein levels and enzymatic activity of MMP-2 and MMP-9 did not have a statistically significant relationship with coronary artery outcome after adjusting for demographic characteristics, and clinical and laboratory features. Although matrix-degrading proteolytic activity was specific for affected mice and localized to inflamed coronary artery segments, the enzymatic activity in the systemic circulation of affected and control mice were not different. Similar to affected children, peripheral blood levels of MMP-9 enzymatic activity did not correlate with coronary artery disease in the animal model. Therefore, circulating levels of MMPs known to act locally may not be useful biomarkers of disease. This is especially relevant to enzymatic activity that is tightly regulated at multiple levels including the local tissue environment. (***Pediatr Res* 61: 710–715, 2007**)

KD is the most common cause of vasculitis in children and is now recognized as the leading cause of acquired heart disease in children in the developed world (1). It is characterized by prolonged fever, rash, bilateral nonexudative conjunctival injection, erythema of the lips and oral mucosa, changes in the extremities, and cervical lymphadenopathy (2,3). KD is almost unique among coronary vessel diseases due to its propensity for aneurysm formation. Coronary aneurysms are an important cause of morbidity and mortality. Coronary artery aneurysms occur in up to 25% of untreated patients, and 5% of patients appropriately treated with IVIG and high-dose aspirin (4–6). When adjusted for BSA, the incidence of CAL increases to 25% of treated patients (7).

Standardized treatment protocols are in place for the treatment of acute KD, however, the ability to identify patients predisposed to poor coronary outcome early in the disease course is lacking. The need to improve outcome and tailor therapy is driving the search for biomarkers of coronary disease. The current model of aneurysm formation suggests the involvement of ECM-degrading enzymes (8–10). The most important ECM protein in the vessel wall is elastin. Elastin is extremely stable, and its degradation is almost exclusively through enzymatic digestion. Breakdown of elastin facilitates vessel wall ballooning and results in dilatation leading to aneurysm formation. One family of ECM-degrading proteases implicated in aneurysm formation is the MMPs. MMPs are tightly regulated at the tissue level by endogenous inhibitors, the TIMP family of molecules. In particular, MMP-2 and MMP-9 are proteases with elastolytic activity found in coronary artery aneurysms from fatal cases of KD (11). Expression of MMPs are induced by pro-inflammatory signals, including tumor necrosis factor (TNF)-α (12,13). Immune activation and production of pro-inflammatory cytokines, specifically TNF-α, are central events in acute KD. The relationship between the production and activity of elastolytic MMPs systemically and locally in the coronary vessels is not known. Coronary artery samples are difficult to obtain from children, thus necessitating an animal model of KD. We use a murine model of KD, LCWE-induced coronary arteritis (14–16). This model mimics all the features of KD, including susceptibility in the young, histopathology, time-course of disease, and response to conventional treatment. To address the relationship between elastolytic MMPs and coronary artery disease in KD, we used a two-pronged approach with studies in both mice and man.

The increased elastolytic activity of MMP-2 and MMP-9 is critical to the development of AAA in a rodent model (17). In addition, our own studies in the LCWE model of KD have demonstrated a significantly reduced incidence of coronary aneurysms in animals with deficient MMP-9 activity (unpublished experiments). Some investigators have detected ele-

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Abbreviations: AAA, abdominal aortic aneurysms; **CAL,** coronary artery lesions; **ECM,** extracellular matrix; **IVIG,** intravenous immunoglobulin; **IVMP,** intravenous methylprednisolone; **KD,** Kawasaki disease; **LCWE,** *Lactobacillus casei* cell wall extract; **MMP,** matrix metalloproteinase; **TIMP,** tissue inhibitor of metalloproteinase

vated serum levels of MMP-2 and -9 in KD patients (18–20), as well as in tissues from fatal cases (11). In this study, we examine the relationship between circulating blood levels of elastolytic MMPs and coronary artery outcome in children with KD and in an animal model of KD.

METHODS

Patients. A single-center prospective study of patients diagnosed with KD was performed. The diagnosis of KD was confirmed in all children by a board-certified pediatric rheumatologist based on the presence of ≥5 d of fever plus 4/5 KD criteria: nonpurulent conjunctivitis, oral mucosal changes, cervical lymphadenopathy (≥1.5 cm), rash, and peripheral changes (puffy hands/feet, palmar/plantar erythema) (4). All children had standardized KD assessments at diagnosis and follow-up, including clinical examination, laboratory testing, and echocardiogram. Patients were admitted to hospital and commenced a standardized treatment protocol. This study was approved by the Research Ethics Board at the Hospital for Sick Children, and informed consent was obtained from all participants.

Data collection. All clinical data were prospectively collected using standardized KD data collection forms. Laboratory data including serum hematology, serum chemistry, and sequential echocardiograms were also prospectively collected and independently reviewed. All data were entered into a designated Kawasaki Study database (Microsoft Access study database, Microsoft Corporation, Seattle, WA) using a double data entry verification technique.

Laboratory data. A set of standardized inflammatory markers was obtained at KD diagnosis before IVIG therapy, which included the following laboratory measures: white blood cell count, Hb, platelets, albumin, erythrocyte sedimentation rate, alanine transaminase, IgA, IgM, IgG, and α-1-antitrypsin. All data were included in the analysis.

Echocardiography and definitions. Sequential echocardiograms were performed in each patient at the time of diagnosis, 6 wk and 1 y post-diagnosis. For the left anterior descending, left main and right main coronary arteries, serial echocardiogram measurements of vessel diameters were converted into BSA-adjusted Z-scores using published normal regression equations (7). CAL were defined as coronary artery diameter ≥2.5 SD above the mean for BSA. Aneurysms were defined as a diameter of >4 mm but ≤8 mm. For children under the age of 5, a diameter of >3 mm was considered an aneurysm. Giant aneurysms were defined by a diameter of >8 mm for all age groups. Patients were assigned to groups without CAL (Non-CAL), with CAL (CAL), or with aneurysms (ANEURYSM), as determined by the results of echocardiograms taken at initial diagnosis, 6 wk, and/or 1 y follow-up.

Statistical analysis. Comparison of characteristics between patients in the Non-CAL, CAL, or ANEURYSM groups was performed using χ^2 test and ANOVA as appropriate. Mixed linear regression analysis for repeated measures was used to determine the independent associations of Z-scores with the potential predictive factors being studied. All analyses were performed using statistical software with default settings (SAS version 8, SAS Institute, Cary, NC).

ELISA. All patient blood samples were processed immediately by centrifugation at 700 *g* for 10 min at 4°C. Serum was aliquoted and stored at −80°C until use. Pro- and active MMP-9 protein levels were assayed by the Human MMP-9 Quantikine ELISA Kit as per manufacturer's protocol (Intra-assay CV <3%, inter-assay CV <8%, R&D Systems, Minneapolis, MN). Pro- and active MMP-2 protein levels were quantified using the Human MMP-2 ELISA Kit as per manufacturer's protocol (Intra- and inter-assay CV <12%, Calbiochem, San Diego, CA). ELISA plates were read using a SpectraMAX 250 plate reader (Molecular Devices, Sunnyvale, CA).

Murine model. Coronary arteritis resembling KD was induced in experimental mice using LCWE (15,16). LCWE was prepared as previously described (15,21). Total rhamnose content of LCWE was determined (22), and expressed in mg/mL final concentration in PBS. C57BL/6 mice (4–5 wk) were injected intraperitoneally with 1.0 mg LCWE, or 0.5 mL PBS as a negative control. All animal experiments were approved by the Animal Care Committee at the Hospital for Sick Children Research Institute.

Sample collection. Mice were sacrificed at predetermined time points to reflect the disease course. Blood was collected and immediately centrifuged at 960 *g* for 10 min at 4°C. Serum was aliquoted and stored at −80°C until use. Hearts were isolated and the origin of the coronary vessels identified. The root of the aorta and the coronary vessels together with the base of the heart were separated and homogenized immediately in ice-cold lysis buffer (50 mM Tris pH7.6, 2% SDS, 0.1 mM phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin), and protein extracts were centrifuged at 13,600 *g* for 10 min at 4°C.

Zymography. Total protein content of murine serum and heart extract, and human serum were determined using the DC-Protein Assay (Bio-Rad, Mississauga, Ontario). Equal amounts of total protein (50 μg) were diluted with lysis buffer and resolved through 8% nonreducing SDS-PAGE cross-linked with 0.1% gelatin (Sigma Chemical Co.-Aldrich, St. Louis, MO). Gels were washed in several changes of 2.5% Triton X-100 for 30–60 min and incubated in proteolysis buffer (50 mM Tris pH 8.0, 2.5 mM $CaCl_2$, 0.02% NaN_3) overnight at 37°C. To verify metal-dependent protease activity, replicate gels were incubated in the presence of 20 mM EDTA. Gels were stained (0.25% Coomassie Brilliant Blue R-250, 50% methanol, 10% acetic acid), and destained as appropriate (50% methanol, 10% acetic acid). Areas of gelatinolytic activity were visualized as clear bands against a blue background, and imaged with a Kodak Digital Science camera (Eastman Kodak, Rochester, NY). Internal controls were performed for each gel to ensure consistent analysis.

In situ zymography. Mice were injected with LCWE, or PBS as a negative control, and sacrificed at predetermined time points. Hearts were removed, embedded in OCT medium (Tissue-Tek, Fort Washington, PA), and snap frozen in liquid nitrogen. Cryosections (6 μm) were prepared beginning at the coronary orifice and mounted onto films embedded with gelatin for *in situ* zymographic analysis (MMPs *in situ* Zymo-Film, WakoUSA, Richmond, VA). Films were incubated for 20–24 h at 37°C in a humidified chamber, and air-dried for 30 min. Films were treated with staining solution (1% Amido Black 10B, 70% ethanol, 10% acetic acid) for 15 min and rinsed with water. Gelatinolytic activity was visualized as clear areas against a dark background, and imaged with OpenLab software (Improvision, Lexington, MA).

RESULTS

Patients and clinical features. Forty-six children, 26 boys and 20 girls, were diagnosed with typical KD and included in the study. The mean age at diagnosis was 4.43 y and the median age was 4 y (range 0.45–11.2 y). KD criteria at diagnosis included: fever in 100% (mean 7 d), conjunctivitis in 96%, rash in 87%, oral changes in 87%, peripheral changes in 78%, and lymphadenopathy in 78%. The ANEURYSM group consisted of 6 patients, and the CAL group consisted of 6 patients. The Non-CAL group consisted of 34 patients. There were no significant differences between the three groups in regards to their demographic or clinical features including: male-to-female ratio, days of fever, conjunctivitis, rash, oral changes, peripheral changes and lymphadenopathy (Table 1), except for age, which differed between the Non-CAL and CAL groups ($p = 0.008$).

Response to treatment. The standardized KD treatment protocol used at our institution included IVIG (IVEEGAM 2g/kg, to a maximum of 70 g) plus high-dose aspirin (80–100 mg/kg/d) until afebrile for 24 h. This was followed by low-dose aspirin, 3–5 mg/kg/d, until the 6-wk follow-up echocardiogram. Treatment for resistant fever or fever recrudescence (>24 h after end of IVIG infusion) was a second dose of IVIG at 2 g/kg. Failure of the second IVIG treatment lead to treatment with intravenous methylprednisolone (IVMP) (30 mg/kg/d, to a maximum of 1.0 g) given for three consecutive days. This was followed by oral prednisone (2 mg/kg/d) for 1 wk tapered to 0 over the next 2 wk, unless persistent fever or fever recrudescence required prolonged use. After a single dose of IVIG, 78% of patients (36/46) responded, however, 22% of patients (10/46) required a second dose of IVIG. Two of these 10 patients (4% of the total group) needing re-treatment required IVMP therapy in addition to multiple IVIG doses. The re-treatment rates between the three patient groups did not differ statistically (Table 2), though it should be noted that statistical power was limited due to the small sample size.

Table 1. *Patient characteristics*

	Total	Non-CAL	CAL	ANEURYSM
No.	46	34	6	6
Mean age (y)	4.43 ± 2.68*	3.97 ± 2.52	7.04 ± 2.14**	4.43 ± 2.93†‡
Median age (y)	4.00 (0.45–11.20)	3.39 (0.45–10.61)	6.37 (5.25–11.20)	4.06 (1.28–8.25)
Male:female	26:20	16:18	4:2	6:0
Duration of fever (d)	7.00 ± 2.89	6.85 ± 2.96	7.17 ± 2.79†	7.67 ± 3.01†‡
Conjunctivitis	44/46 (96%)	33/34 (97%)	5/6 (83%)†	6/6 (100%)†‡
Oral changes	40/46 (87%)	30/34 (88%)	6/6 (100%)†	4/6 (67%)†‡
Rash	40/46 (87%)	29/34 (85%)	6/6 (100%)†	5/6 (83%)†‡
Peripheral changes	36/46 (78%)	25/34 (74%)	6/6 (100%)†	5/6 (83%)†‡
Lymphadenopathy	36/46 (78%)	27/34 (79%)	4/6 (67%)†	5/6 (83%)†‡

* Where applicable, data are expressed as mean ± SD.
** $p = 0.008$, compared with Non-CAL group.
† NS, compared with Non-CAL group.
‡ NS, compared with CAL group.

Table 2. *Response to treatment*

	Total	Non-CAL	CAL	ANEURYSM
Response to IVIG	36/46 (78%)	27/34 (79%)	6/6 (100%)*	3/6 (50%)*†
Retreatment rate	10/46 (22%)	7/34 (21%)	0/6 (0%)*	3/6 (50%)*†
Two doses of IVIG only	8/10	5	0	3
Two doses of IVIG + IVMP	2/10	2	0	0

* NS, compared with Non-CAL group.
† NS, compared with CAL group.

Coronary artery lesions. CAL, defined as BSA-adjusted Z-scores ≥2.5 SD, were seen on echocardiograms in 6/46 of the KD patients, comprising 13% of the entire study population. Aneurysms (vessel diameter >4 mm) were seen in 6 children (13% of the patient population), with none meeting the criteria of a giant aneurysm (>8 mm).

Protein and activity measures of MMP-2 and MMP-9. An ELISA was used to determine the levels of MMP-2 and MMP-9 protein in the serum of patients with or without coronary artery abnormalities. Identical serum samples were also used for analysis of MMP-2 and MMP-9 enzymatic activity by gelatin zymography. Samples were normalized by total protein, and band intensity of enzymatic activity was quantified by OD. Serum protein levels and enzymatic activity of MMP-9 and MMP-2 did not differ statistically between the Non-CAL, CAL, or ANEURYSM Groups (Fig. 1 and 2, respectively).

Factors affecting coronary outcome. Factors associated with normalized coronary dimensions over time were sought in multi-variable mixed regression analysis. The following variables were tested: demographic information including gender and age at diagnosis; KD clinical features (diagnostic criteria) including duration of fever, rash, oral-mucosal changes, cervical lymphadenopathy, nonpurulent conjunctival injection, and peripheral changes; and standard laboratory

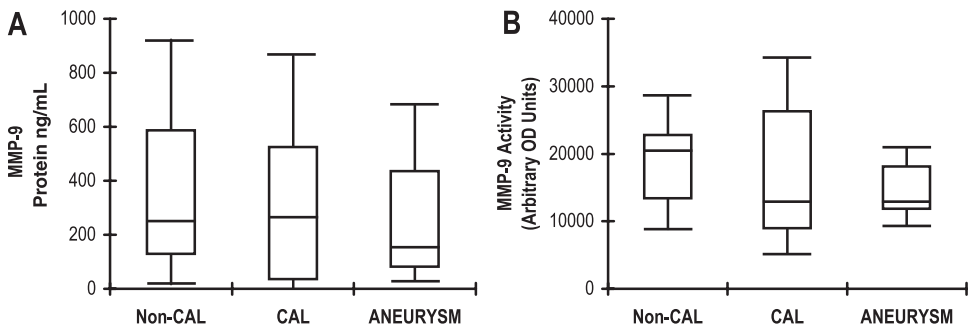

Figure 1. MMP-9 protein and enzymatic activity do not differ with coronary outcome. Serum was processed from Non-CAL (n = 34), CAL (n = 6), and ANEURYSM (n = 6) patients and assayed for total MMP-9 protein using ELISA (*A*, NS). Line indicates median. Corresponding MMP-9 enzymatic activity in identical serum samples was assessed by gelatin zymography (*B*, NS). Bands indicating MMP-9 proteolytic activity were quantified by OD and expressed as OD units.

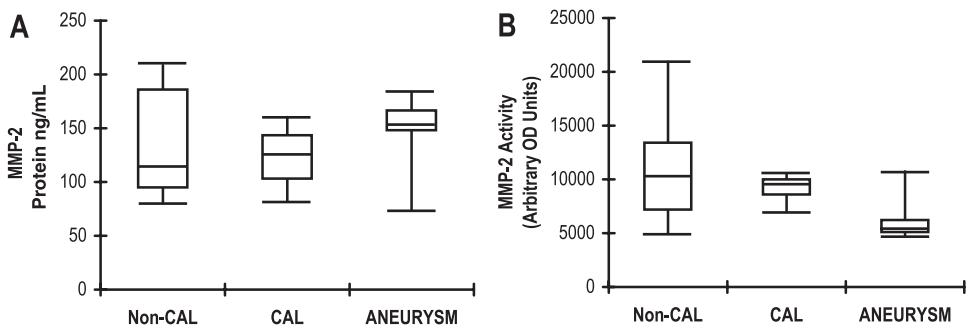

Figure 2. MMP-2 protein and enzymatic activity do not differ with coronary outcome. Serum was processed from Non-CAL (n = 34), CAL (n = 6), and ANEURYSM (n = 6) patients and assayed for total MMP-2 protein using ELISA (*A*, NS). Line indicates median. Corresponding MMP-2 enzymatic activity in identical serum samples was assessed by gelatin zymography (*B*, NS). Bands indicating MMP-2 proteolytic activity were quantified by OD and expressed as OD units.

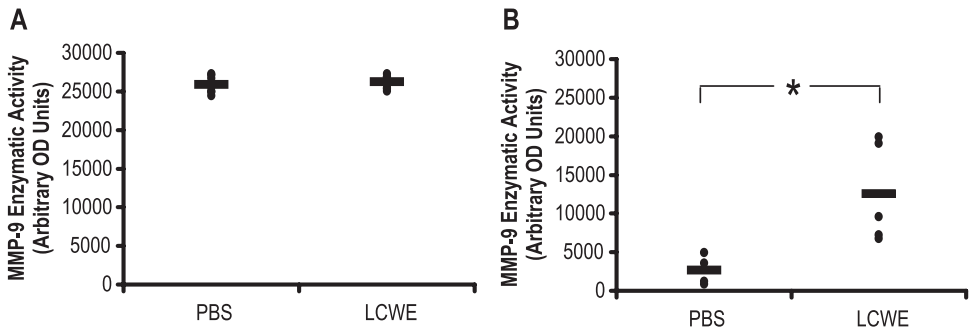

Figure 3. Elevated MMP-9 activity at the target tissue, but not systemically. Serum (*A*) and heart protein extracts (*B*) from PBS- ($n \geq 4$) or LCWE-injected ($n \geq 5$) wild-type mice were collected and MMP-9 activity was assessed by gelatin zymography. Bands indicating MMP-9 proteolytic activity were quantified and expressed as OD units. Line indicates mean. *$p = 0.023$.

features including erythrocyte sedimentation rate, C-reactive protein, white blood cell count, Hb, platelets, albumin, alanine transaminase, IgA, IgM, IgG, and α-1-antitrypsin. In addition, experimental laboratory assays including measures of protein levels of MMP-2 and MMP-9 in pretreatment serum samples and their corresponding enzymatic activity were examined. Multivariate regression analysis of repeated measures determined the impact of these multiple variables on coronary artery diameter regression over time. Regression of coronary artery vessel diameter over time was the continuous outcome variable. Determining the impact of variables on vessel regression was an additional approach to further expand the search for independent risk factors of CAL in our KD population under study.

Six variables had a statistically significant relationship to coronary artery Z-scores: gender, polymorphonuclear cell count, alanine transaminase, peripheral changes, date of echocardiogram, and number of KD criteria. The first five variables increased with Z-scores. However, the sixth variable, number of KD criteria, had an inverse relationship with the Z-score value. Once adjusted for the main variables, the MMP-2 and MMP-9 serum levels did not have a statistically significant relationship with Z-scores ($p = 0.87$ and $p = 0.84$, respectively, data not shown). Further analysis of the data using the log of the protein concentration of MMP-2 and MMP-9 did not significantly affect the p values ($p = 0.84$ and $p = 0.98$, respectively, data not shown).

Relationship between serum and coronary artery expression of MMP-9. We used a well-established model of KD, LCWE-induced coronary arteritis in mice (14–16), to address the relationship of systemic and local events during evolution of coronary artery disease. The LCWE model reflects human KD in its time course, pathology, susceptibility in the young, and response to IVIG therapy. To assess the levels of MMP-9 activity in both the systemic circulation and the affected tissues, gelatin zymography was performed using serum and heart protein extracts from mice injected with LCWE, or PBS as a negative control. Comparable levels of serum MMP-9 activity were found between PBS- and LCWE-injected animals (Fig. 3*A*). However, at the target organ, MMP-9 activity levels in LCWE-injected mice were not only more variable, but significantly higher than controls ($p = 0.023$, Fig. 3*B*). These data demonstrate that circulating levels of MMP-9 activity have no association with those found in the heart, and further suggests that serum MMP-9 is not an accurate marker for local disease at the target organ.

Enzymatic activity in affected coronary arteries. Similar to human KD, LCWE induces a vasculitis that predominantly affects the coronary vessels (15,16,23). To determine enzymatic activity at the target organ, heart cryosections were prepared and *in situ* zymography was performed. Enzymatic activity as visualized by clear areas against a dark background was seen only surrounding the coronary arteries of LCWE-injected animals (Fig. 4*B*). Another field demonstrating myocardium from the identical LCWE-injected animal did not display any evidence of enzymatic digestion (Fig. 4*C*), and resembled controls (Fig. 4*A*). These results indicated that elevated ECM-degrading enzymatic activity was not only

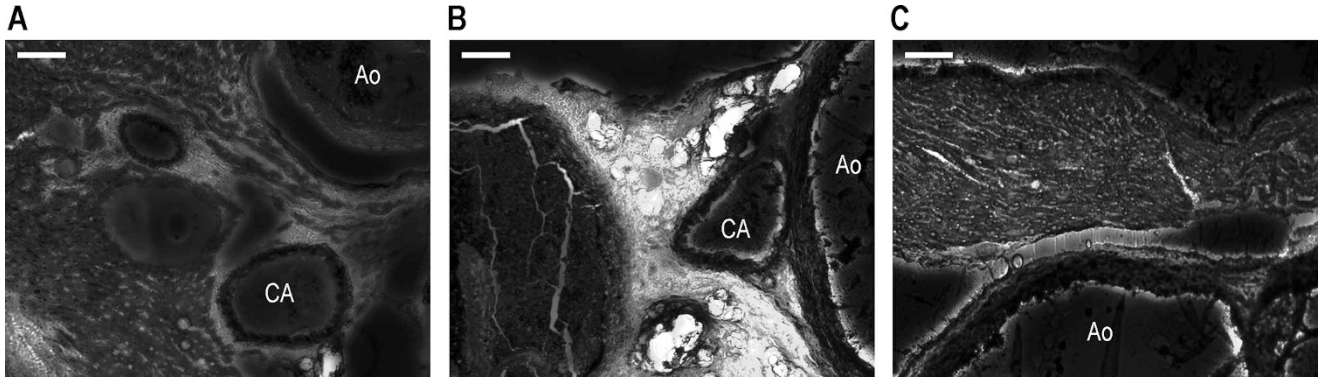

Figure 4. Localized ECM-degrading enzymatic activity at affected coronary arteries. Wild-type mice were injected with PBS (*A*) or LCWE (*B*, *C*) and heart cryosections were prepared 28 d post-disease induction. Cryosections were mounted onto films embedded with gelatin for *in situ* zymographic analysis. Clear areas of digestion, indicating enzymatic activity, were visualized against a dark background (*Ao,* aorta; *CA,* coronary artery). Panel *C* depicts another field containing myocardium from the identical mouse as shown in panel *B*. Bar indicates 50 μm.

specific for disease, but was highly localized, found only in the affected regions of the heart, namely surrounding the coronary arteries.

DISCUSSION

KD is a multisystem vasculitis affecting medium and small vessels. The damage caused by inflammation predominantly affects the coronary circulation leading to aneurysm formation. Coronary artery aneurysms as a consequence of KD are now the leading cause of acquired heart disease in children from the developed world (1). In this article, we report that expression of elastolytic enzymes are specific to affected mice and enzymatic activity is localized to affected coronary arteries, but there is no relationship with circulating blood levels. Specifically, the levels of MMP-2 and MMP-9 in the peripheral circulation have no correlation with coronary outcome. Therefore, circulating levels of MMPs do not reflect local enzymatic activity in the affected tissue and are not good biomarkers of disease activity or outcome.

Elastin breakdown with resultant medial destruction and arterial dilatation is the hallmark of aneurysm formation (8). MMPs are well entrenched in the literature as mediators of ECM degradation leading to AAA formation. In particular, the elastolytic enzymes, MMP-2 and MMP-9, have been implicated. Increased MMP-2 and MMP-9 expression was detected in human AAA (10). MMP-2 may be more important in small early aneurysms, while increased MMP-9 expression becomes more prominent as the inflammatory infiltrate increases and the aneurysm enlarges (13,24). Local over-expression of TIMP-1, the tissue inhibitor for MMP-9, prevents aneurysmal degeneration and rupture in a rat model of aortic aneurysms (25). This phenotype is echoed by experiments in MMP-9-deficient mice, which are protected from experimentally induced AAA, thus confirming an important role for MMP-9 in elastin degradation (9).

In our group of children with typical KD, the demographic (age and gender) characteristics, clinical and laboratory features, response to treatment, and coronary outcome are similar to other published reports (7,26,27). To address the predictive value of MMP-2 and MMP-9 as biomarkers of coronary outcome in these children with typical KD, a multivariate regression analysis of repetitive measures was conducted. Regression of coronary vessel diameter over time (determined by BSA-adjusted Z-scores) was the continuous outcome variable. Normalizing dimensions for body size and then using statistical methods that can examine changes over time allows for a clearer picture regarding persistence, regression, and resolution of CAL. Furthermore, potentially confounding predictive factors of coronary outcome can also be examined and accounted for to assess the predictive value of MMP-9 and MMP-2. Our results showed that there was no significant relationship between pretreatment levels of total MMP-9 or total MMP-2 protein and coronary outcome. Additionally, there was no significant association of coronary outcome with enzymatic activity of MMP-9 and MMP-2.

The studies pointing to the role of the MMPs family of enzymes in aneurysm formation in KD are growing (11), but the potential value of these molecules as biomarkers for disease diagnosis or prognosis has not been realized. Although studies have shown that pro-MMP-9 protein levels are elevated in children with acute KD compared with both febrile and afebrile controls, the enzymatic activity of MMP-9, though readily detected, was not significantly different from controls (20). Furthermore, despite the fact that MMP-9 was elevated in affected hearts in our murine model of KD, there was no correlation between MMP-9 circulating levels and enzymatic activity in affected coronary arteries (Fig. 3).

The increased proteolytic activity detected in the LCWE model of KD was specific for disease, found only around affected coronary arteries and localized to areas demonstrating elastin breakdown (Fig. 4*B*). This was in accord with data from cases of fatal acute KD, which showed prominent expression of both MMP-2 and MMP-9 at the site of coronary artery aneurysms (11). Interestingly, MMP-9 expression was specific to children with KD, found in both coronary artery aneurysms and in unaffected arterial segments in children with KD, but not in non-KD heart tissue. MMP-2 was less specific, with expression found in vascular tissue from both children with KD and those without. Despite evidence from our work and in the literature supporting the importance of MMP-2 and MMP-9 in local proteolytic activity and development of coronary lesions, this local activity is not reflected in the systemic circulation. This may be due, in part, to the tight regulation of MMPs activity at the tissue level.

Local MMPs enzymatic activity is an important pathogenic event leading to blood vessel wall destruction. MMPs inhibitors, including members of the tetracycline family, have been used in humans and in experimental animals to block the enzymatic activity of MMPs. In adults with AAA, tetracycline is effective in inhibiting local MMPs activity (28,29). Mice given either systemic tetracycline in the drinking water (9) or by local perivascular infusion have not only decreased MMPs activity but also demonstrated a decrease in aneurysm formation (30). Interestingly, the neutrophil elastase inhibitor, ulinastatin, also has anti-MMP properties and is used in the treatment of recalcitrant KD patients in Japan (31).

In summary, although MMP-9 and MMP-2 may play an important role in the formation of CAL at the site of tissue damage, circulating peripheral blood protein levels and their corresponding enzymatic activity have no relationship with coronary artery damage. Circulating levels of molecules known to act locally may not be useful biomarkers of disease. This is especially relevant to enzymatic activity that is tightly regulated at multiple levels including the local tissue environment. Searching for breakdown products of MMPs proteolytic activity in the peripheral blood may be another avenue to identify a surrogate marker of local MMPs activity and disease activity.

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