S100 Proteins in the Pathogenesis of Kawasaki Disease*

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Kawasaki disease (KD) is an acute, self-limited vasculitis of infants and children, and its successful treatment depends on timely diagnosis. This vasculitis of unknown etiology, which is now the most common form of acquired heart disease in children in the U.S. and Japan (1,2), presents with clinical signs that include fever, rash, conjunctival injection, edema and erythema of the extremities, and oropharyngeal erythema (3). Although the acute illness is self-limited, one in four untreated children will develop permanent coronary artery damage with aneurysms or ectasia as a consequence of the vasculitis (4). The acute inflammation can be abrogated in the majority of patients with a single dose of intravenous immunoglobulin (IVIG) plus high-dose aspirin, which reduces the aneurysm rate to 3% to 5% if administered within the first 10 days after fever onset (5). Unfortunately, without a specific laboratory test, diagnosis of KD relies on the recognition of the clinical signs, which may appear and disappear over a period of many days. Thus, patients continue to be misdiagnosed and suffer preventable morbidity and mortality because of failure to recognize the condition and to administer therapy in a timely manner.

Although the combination of IVIG and aspirin therapy is highly effective, 10% to 15% of patients will be refractory to this treatment regimen and will have persistent fever as a manifestation of ongoing inflammation (6). These patients are at highest risk for the development of coronary artery damage. Potential new therapeutic approaches with anti-tumor necrosis factor-alpha monoclonal antibodies are being tested in clinical trials in KD patients (7). Such new therapies may provide the clinician with an expanded armamentarium and make the early identification of patients at risk for failure to respond to IVIG more relevant.

Transthoracic echocardiography is used to classify patients as having normal, dilated, or aneurismal coronary arteries (8). The long-term sequelae of the coronary artery aneurysms include ischemic heart disease and myocardial infarction. Several scoring systems have been devised to identify patients at highest risk for development of coronary artery aneurysms, but none have been validated in prospective studies in non-Asian patients (9). Patients at highest risk for coronary artery damage may be candidates for more aggressive antiplatelet and anti-inflammatory therapy. Thus, being able to predict both failure to respond to IVIG and increased risk of aneurysms would have important therapeutic implications for these patients.

In the absence of knowing the etiologic agent or agents for KD, biomarker discovery has been an active area of investigation. The goals have been to identify proteins in the blood or urine that would help to differentiate KD from other childhood rash/fever illnesses and to predict both response to IVIG therapy and coronary artery damage. In the paper by Hirono et al. (10) in this issue of the Journal, 2 calcium-binding proteins in the S-100 family, (MRP)-8 and MRP-14 (e.g., S100A8 and A9) were tested as potential biomarkers for KD and coronary artery sequelae. These proteins form heterodimers and are secreted by neutrophils and monocytes in response to inflammatory signaling cascades (Fig. 1). The MRP8/MRP14 heterodimer binds to microvascular endothelial cells and may participate in the genesis of a proinflammatory and prothrombotic state during systemic vasculitis (11,12). Specifically, these proteins regulate adhesion of neutrophils and monocytes to endothelial cells and are implicated in their transmigration into the vessel wall (13). Serum levels of the heterodimer MRP8/MRP14 correlate with disease activity in conditions characterized by systemic inflammation, including juvenile rheumatoid arthritis (14). Another member of the S100 family, S100A12, is also released by neutrophils and monocytes, binds to the receptor for advanced glycation end products (RAGE) on endothelial cells and leukocytes, and induces cell activation and cytokine production through the nuclear factor-kappa-B signaling pathway (15,16). Transcript and protein levels of all 3 of these S100 proteins are increased in circulating leukocytes and in serum during the acute phase of KD (12,17–20).

In the new study reported, the authors (10) measured levels of MRP8/MRP14 in serum, mRNA levels in circulating neutrophils and monocytes, and the presence of the heterodimer bound to circulating, shed endothelial cells during the acute and convalescent stages of KD. As in previous studies, serum levels of MRP8/MRP14 were markedly elevated in KD patients compared with healthy controls. Unfortunately, febrile controls with non-KD rash/fever illnesses were not included, so no conclusion can be drawn regarding the possibility of MRP8/MRP14 heterodimers as biomarkers for KD. Of the 61 KD patients, 16 (26.2%) were IVIG nonresponders. Although patients who had a response to IVIG had a clear and progressive decrease in heterodimer levels after IVIG administration, patients who had persistent or recrudescent fevers had no coherent pattern of heterodimer decrease, and levels actually increased in some patients. These nonresponder patients also had an increase in granulocyte mRNA levels for MRP8 and

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MRP14 at 2 weeks after IVIG infusion. Elevated levels of heterodimer in acute KD patients before therapy followed by markedly decreased levels after IVIG infusion have also been reported by other groups (12,17). Abe et al. (17) used microarray and kinetic polymerase chain reaction to quantify transcripts of MRP8 and MRP14 and measured heterodimer levels in the plasma of KD patients and febrile controls. They found a reduction in peripheral blood mononuclear cell and monocyte transcript abundance as well as plasma levels of MRP8 and MRP14 after IVIG treatment. They also noted persistently high levels of heterodimer in the plasma of four KD patients in whom coronary artery aneurysms developed. Ebihara et al. (19), using kinetic polymerase chain reaction in paired acute and convalescent whole-blood samples from 10 KD patients, also reported elevated pretreatment transcript levels of these S100 proteins. Viemann et al. (12) found the same rapid decrease in heterodimer levels after IVIG infusion and used immunohistochemistry to demonstrate MRP8/MRP14-positive neutrophils adherent to the endothelium of myocardial vessels from three KD autopsies. This group also showed MR8/MR14 heterodimers bound to the endothelial cells. This is consistent with the finding by Hirono et al. (10) of high numbers of circulating shed endothelial cells with bound MRP8/MRP14 in patients with coronary artery abnormalities at 2 weeks after treatment. In vitro experiments have shown that human microvascular endothelial cells incubated with purified MRP8/MRP14 heterodimers upregulate expression levels of genes involved in platelet aggregation and inflammation and downregulate expression of genes involved in endothelial integrity (12). Taken together, these observations support the hypothesis that MRP8/MRP14 heterodimers participate directly in the pathogenesis of the coronary artery vasculitis in KD and contribute to endothelial cell damage and transmigration of leukocytes into the arterial wall.

Unfortunately, elevated numbers of heterodimer-positive circulating endothelial cells cannot be used to predict coronary artery damage because pretreatment levels were low and the increase in circulating levels coincided with the appearance of coronary artery dilatation by echocardiogram. Although MRP8/MRP14 heterodimer levels will not help predict responsiveness to IVIG therapy or the development of coronary artery lesions, these proteins are likely to be important in disease pathogenesis and further investigation into their role in promoting inflammation and endothelial cell damage in KD is warranted.

**Figure 1.** Proposed role of S100 proteins (myeloid-related protein [MRP]-8, MRP-14, and S100A12) in the genesis and maintenance of the vasculitis in Kawasaki disease. (Top) Tumor necrosis factor (TNF)-alpha and other proinflammatory cytokines activate endothelium and lead to expression of carboxylated N-glycans. Activated neutrophils and monocytes secrete MRP-8/MRP-14 heterodimers, which bind to the carboxylated N-glycans and heparin sulfate on the endothelial cell surface. Leukocytes also secrete S100A12, which binds to the receptor for advanced glycation end products (RAGE) expressed on endothelial cells, lymphocytes, and macrophages. This receptor signals through the nuclear factor-kappa-B pathway and induces expression of many proinflammatory molecules. (Bottom) The net result of S100 protein binding is platelet aggregation and adherence to endothelium, increased expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, adhesion of neutrophils and monocytes, loosening of endothelial cell junctions, and trafficking of inflammatory cells across the endothelial cell barrier. Illustration by Rob Flewell.

**REFERENCES**


