Calgranulins May Contribute Vascular Protection In Atherogenesis
Carolyn L. Geczy, PhD; Yuen Ming Chung, BSc; Yuka Hiroshima, PhD

S100A8, S100A9 and S100A12 are considered proinflammatory mediators of atherosclerosis. Known as calgranulins, they are major components of neutrophils and are upregulated in macrophages and foam cells. They influence leukocyte recruitment, and may propagate inflammation by binding TLR4 and/or receptor for advanced glycation endproducts (RAGE). However, the receptors for calgranulins remain an enigma; we have no evidence for TLR4 or RAGE activation by S100A8 or S100A12. Moreover, gene regulation studies suggest antiinflammatory functions for S100A8 and emerging reports indicate pleiotropic roles. Unlike S100A9, S100A8 effectively scavenges oxidants generated by the myeloperoxidase system in vivo, forming novel thiol modifications. S100A8 is also readily S-nitrosoylated, stabilizing nitric oxide and transporting it to hemoglobin. S100A8-SNO reduces leukocyte transmigration in the vasculature. S-glutathionylation of S100A9 modifies its effects on leukocyte adhesion. Both S100A8 forms inhibit mast cell activation, at least partially by scavenging reactive oxygen species required for signaling. Conversely, S100A12 activates and sequesters mast cells. However S100A12 suppresses proinflammatory cytokine induction by SAA-activated monocytes and macrophages, and inhibits matrix metalloprotease activity. We propose that the abundance and types of cells expressing calgranulins in particular microenvironments, their relative concentrations and post-translational modifications may have distinct functional outcomes, including those that are protective, at different stages of atherogenesis. (Circ J 2014; 78: 271–280)

Key Words: Atherosclerosis; Mast cells; Neutrophils; Redox; S100 proteins

Inflammation and Atherogenesis
Inflammation underpins the pathogenesis of atherosclerosis and innate and cell-mediated immune responses are major contributors, promoting early vascular changes and leukocyte recruitment into the vessel intima (reviewed in Legein et al1 and Libby). Today, there is an important new focus on the roles of neutrophils and mast cells (MC) in atherogenesis. Neutrophil functions are being elucidated (reviewed in Soehnlein and Simon & Zidar). These are the main type of leukocyte interacting with atherosclerotic endothelium where there is ongoing recruitment and accumulation within shoulder regions, with macrophages. Neutrophils in human endarterectomy specimens localize to rupture-prone culprit lesions. Elevated numbers of circulating neutrophils are associated with hyperlipidemia, correlate with plaque size and indicate risk of cardiovascular mortality. Importantly, these neutrophils appear activated, producing high levels of reactive oxygen species (ROS) and releasing myeloperoxidase (MPO), key mediators of oxidative damage. MPO that has localized to the endothelial basement membrane contributes to endothelial dysfunction and plaque rupture by generating the powerful oxidant, HOCl. In addition, neutrophil proteases may promote matrix destruction, and mediators such as azurocidin and α-defensins may facilitate monocyte recruitment, phagocytosis and foam cell formation. Some neutrophil mediators potentially influencing atherogenesis are summarized in Figure 1. Activated neutrophils release their nuclear content, forming neutrophil extracellular traps (NETS) that bind and localize many mediators. These are found in atherosclerotic lesions and may contribute to chronicity and thrombosis. However, neutrophils also have protective functions, particularly in neointima formation and endothelial cell recovery, some mediated by cathelicidin.

This review focuses on some of the roles of the calgranulins, S100 proteins S100A8, S100A9 (also known as MRP8 and MRP14, respectively) and S100A12 that are abundant in neutrophils and associated with atherogenesis, with emphasis on protective aspects, particularly in antioxidant defense and modulating MC and monocyte activation.

S100 Calgranulins
The calgranulins comprise approximately 40% of the neutrophil cytoplasm. Many studies associate elevated levels of the S100A8/A9 complex (known as calprotectin because of its antimicrobial and antiinvasive properties [reviewed in Hsu et
al[11]) in the circulation with clinical activity of numerous inflammatory diseases.12-14 Unlike S100A8 and S100A9, there is no S100A12 in rodent genomes; human S100A12 lies between S100A8 and S100A9 in a subcluster of 100 genes on chromosome 1q21 and is structurally similar to these.15 S100 proteins are small hydrophobic Ca2+-binding proteins released as a result of necrosis, or are actively secreted,16 and are components of NETS.17 S100A8 and S100A9 interact noncovalently, forming Ca2+-dependent complexes in solution,18 possibly the preferred form in neutrophils. However, they are not always coexpressed and have independent functions. For example, only S100A8 is induced in murine macrophages by TLR agonists19,20 and S100A8 plays a critical role in embryo development before S100A9 is expressed.21 The calgranulins are also constitutively expressed in myeloid-derived suppressor cells and monocytes, but not tissue macrophages.

The pattern of induction of S100A8 in macrophages indicates antiinflammatory properties. Induction by lipopolysaccharide (LPS) or dsRNA is complex, and interleukin (IL)-10-dependent.19,22 In keeping with a role in resolution of inflammation, we find that classically activated macrophages stimulated with interferon-γ (IFN-γ) plus LPS (M1 type) do not express S100A8 whereas inducible nitric oxide synthase (iNOS) is highly upregulated.23 On the other hand, macrophages differentiated to the M2 type (mediate resolution), express S100A8 without further stimulation [Hiroshima & Geczy, unpubl. data]. Moreover, glucocorticosteroids (GC) potentiate IL-10-dependent gene induction by LPS,24 and S100A8 is elevated in the lungs of mice treated with LPS and dexamethasone compared with LPS alone, even though proinflammatory mediators are reduced,24 supporting a protective phenotype. Dendritic cells (DC) differentiated with granulocyte-macrophage colony stimulating factor (GM-CSF) express high levels of S100A8 and S100A9 compared with M-CSF differentiated macrophages and, like macrophages,25 LPS plus interferon (IFN)-γ reduces expression.26

Several nonmyeloid cell types express calgranulins following activation, including microvascular endothelial cells (EC);26 GC are similarly synergistic.20 S100A8 is expressed in microvessels and capillaries in delayed-type hypersensitivity lesions,26 and in neovessels, but not arteries or large vessels, in human atheroma.27 Fibroblasts28 and epithelial cells express these proteins after appropriate stimulation and ROS promote induction in some circumstances.29 Mild skin stressors also upregulate S100A8 and S100A9 and roles in wound repair and oxidant defense are proposed.29,30 However, compared with macrophages, we find only very low amounts secreted by nonmyeloid cells.

S100A12 expression is somewhat more restricted and IL-10-independent. It is induced in monocytes and macrophages by LPS, tumor necrosis factor alpha (TNF-α)31 and IL-6.32

Calgranulins in Diseases Predisposing to Cardiovascular Risk

High circulating levels of S100A8/A9 are reported in patients suffering from acute and chronic inflammatory disorders, including conditions increasing cardiovascular risk. Concentrations frequently correlate with disease course and/or severity and with clinical scores, and decline following therapy (reviewed in Foell et al[12]). Elevated calgranulin levels in certain chronic inflammatory conditions are suggested to contribute to atherogenesis. These include periodontitis; Porphyromonas gingivalis LPS promotes S100A8/A9 release from human neutrophils via a CD14-TLR2-NF-κB pathway.33 Arthropathies, including rheumatoid arthritis (RA) and systemic lupus erythe-
matisus (SLE) are also independent risk factors for cardiovascular disease (CVD), with greater effects than most traditional risk factors, and calgranulins in the circulation, within inflamed synovium and/or synovial fluid, are elevated in these conditions. The long-term effects of treating RA patients with TNF-α antagonists indicate strong correlations between improved aortic stiffness and circulating levels of S100A8/A9 and C-reactive protein (CRP). Elevated S100A8/A9 and S100A12 in serum may indicate severe disease and CVD in SLE.

Levels of S100A12 mRNA in peripheral leukocytes, and protein in serum from chronic kidney disease patients with CVD are elevated and higher in hemodialysis patients with atherosclerosis than in controls, possibly reflecting a systemic inflammatory state. A cross-sectional study suggests S100A12 involvement in the progression of atherosclerosis as a complication of endstage renal disease. S100A12 levels correlate positively with maximum carotid intima-media thickness, prevalence of peripheral arterial disease, carotid atherosclerosis and/or plaque formation, and predict CVD-related mortality.

Calgranulins in CVD
The calgranulins are expressed in macrophages, foam cells and neovessels in human atheroma, particularly in areas of plaque rupture. Infiltrating neutrophils and macrophages express high amounts of S100A8/S100A9 at sites of acute myocardial infarction (MI). S100A9 associates with areas of calcification and is a proposed marker for imaging high-risk, rupture-prone plaque.

Transcriptional profiling indicated that S100A9 is increased in the platelets from patients with acute plaque rupture and MI. Plasma S100A8/A9 levels, proposed to predict rates of MI, stroke and cardiovascular death in survivors and apparent MI, are elevated in serum S100A8/A9 levels positively correlate with peak neutrophil burst and stabilize intracellular glutathione and NO levels, and neovessels in human atheroma, suggesting altered MMP-9 function in vivo. Backcrosses of S100A12 transgenic mice with hyperlipidemic ApoE-null mice develop somewhat larger plaques, with larger calcified areas than do wild-type/ApoE−/− mice, although S100A12 alone was insufficient to promote vascular changes. Enhanced oxidative stress contributes to vascular calcification and S100A12, possibly via RAGE-driven ROS generation, may increase expression of osteoblastic genes that facilitate calcification. Moreover, S100A12 promotes apoptosis of aortic SMC, and its expression may generate necrotic bodies, forming a nidus for calcification.

On the other hand, S100A12 is a potent inhibitor of matrix metalloproteases (MMP)-2 and -9, by virtue of its ability to chelate Zn2+ from the active sites of these proteases. An antibody specifically reacting with the S100A12-Zn2+ complex has been developed, which may be useful in the treatment of atherosclerosis.
Extracellular Functions and Receptors of Calgranulins

The extracellular proinflammatory functions of calgranulins have been extensively reviewed. These are chemotactic for leukocytes, albeit at very low concentrations, and influence leukocyte adhesion and transmigration but do not activate these cells. Some extracellular functions attributed to S100A8 are summarized in Figure 3. S100A9 modulates leukocyte migration and adhesion and leukocyte recruitment into inflamed tissue is generally reduced in S100A9−/− mice. The calgranulins have been reported to induce proinflammatory cytokines in monocytes/macrophages and are implicated in promoting EC adhesion and dysfunction, affecting SMC proliferation. S100A8 and S100A9 at physiological levels have neutrophil-repulsion activity and reduce the spontaneous and stimulated neutrophil oxidative burst, possibly mediated by adenosine metabolites.

The calgranulin receptors remain an enigma. Structural studies suggest complex multireceptor interactions, or binding of specific ligands on different target cells. S100A8 and S100A9 functions may be dependent or independent of complex formation, whereas S100A8 and S100A12 chemotactic functions reside within the divergent hinge domains. Affinity of S100A12 for the V domain of RAGE increases >1,000-fold in the Ca2+- or Zn2+-bound hexameric states. Only S100A9 with Ca2+ and Zn2+ has high affinity for RAGE; S100A8 has virtually none, and S100A8/A9 binding is relatively weak. Additional putative receptors include N-glycans (glycosylated RAGE) and carboxylated N-glycans, a G-protein-coupled receptor, scavenger receptors (reviewed in Goyette & Geczy).

Discrepancies exist between laboratories concerning TLR4 and RAGE-mediated activation. TLR4 was proposed to bind S100A8, whereas the S100A8/A9 complex was inactive; S100A9 did not activate TLR4 and inhibited S100A8-mediated activation, but enhanced cytokine induction by LPS. In contrast, TLR4 is implicated in monocyte and DC activation by S100A9. Another study showed a requirement for Ca2+ and Zn2+ to achieve high-affinity S100A9 binding to TLR4/MD2, whereas S100A8/A9 binding was less. S100A12 is also recently reported to activate human monocytes via TLR4 rather than RAGE.

In contrast to these results, we have been unable to activate human or murine monocytes/macrophages or EC with any calgranulin. Our preparations were validated for purity and structural integrity, and lacked endotoxin, and were tested at various concentrations using stringent LPS-free culture conditions.

Some Antiinflammatory/Protective Properties of Calgranulins

There is mounting evidence that the calgranulins have pleiotropic functions, some of which may reduce excessive tissue damage and/or facilitate resolution of inflammation. In keeping with mechanisms regulating their expression in macrophages, S100A8/A9 attenuated avridine-induced arthritis in rats, implying that increased local concentrations are protective. Additional putative receptors include N-glycans (glycosylated RAGE) and carboxylated N-glycans, a G-protein-coupled receptor, scavenger receptors (reviewed in Goyette & Geczy).

Discrepancies exist between laboratories concerning TLR4 and RAGE-mediated activation. TLR4 was proposed to bind S100A8, whereas the S100A8/A9 complex was inactive; S100A9 did not activate TLR4 and inhibited S100A8-mediated activation, but enhanced cytokine induction by LPS. In contrast, TLR4 is implicated in monocyte and DC activation by S100A9. Another study showed a requirement for Ca2+ and Zn2+ to achieve high-affinity S100A9 binding to TLR4/MD2, whereas S100A8/A9 binding was less. S100A12 is also recently reported to activate human monocytes via TLR4 rather than RAGE.

In contrast to these results, we have been unable to activate human or murine monocytes/macrophages or EC with any calgranulin. Our preparations were validated for purity and structural integrity, and lacked endotoxin, and were tested at various concentrations using stringent LPS-free culture conditions.

Some Antiinflammatory/Protective Properties of Calgranulins

There is mounting evidence that the calgranulins have pleiotropic functions, some of which may reduce excessive tissue damage and/or facilitate resolution of inflammation. In keeping with mechanisms regulating their expression in macrophages, S100A8/A9 attenuated avridine-induced arthritis in rats, implying that increased local concentrations are protective. Additional putative receptors include N-glycans (glycosylated RAGE) and carboxylated N-glycans, a G-protein-coupled receptor, scavenger receptors (reviewed in Goyette & Geczy).

Discrepancies exist between laboratories concerning TLR4 and RAGE-mediated activation. TLR4 was proposed to bind S100A8, whereas the S100A8/A9 complex was inactive; S100A9 did not activate TLR4 and inhibited S100A8-mediated activation, but enhanced cytokine induction by LPS. In contrast, TLR4 is implicated in monocyte and DC activation by S100A9. Another study showed a requirement for Ca2+ and Zn2+ to achieve high-affinity S100A9 binding to TLR4/MD2, whereas S100A8/A9 binding was less. S100A12 is also recently reported to activate human monocytes via TLR4 rather than RAGE.

In contrast to these results, we have been unable to activate human or murine monocytes/macrophages or EC with any calgranulin. Our preparations were validated for purity and structural integrity, and lacked endotoxin, and were tested at various concentrations using stringent LPS-free culture conditions.

Some Antiinflammatory/Protective Properties of Calgranulins

There is mounting evidence that the calgranulins have pleiotropic functions, some of which may reduce excessive tissue damage and/or facilitate resolution of inflammation. In keeping with mechanisms regulating their expression in macrophages, S100A8/A9 attenuated avridine-induced arthritis in rats, implying that increased local concentrations are protective. Additional putative receptors include N-glycans (glycosylated RAGE) and carboxylated N-glycans, a G-protein-coupled receptor, scavenger receptors (reviewed in Goyette & Geczy).

Discrepancies exist between laboratories concerning TLR4 and RAGE-mediated activation. TLR4 was proposed to bind S100A8, whereas the S100A8/A9 complex was inactive; S100A9 did not activate TLR4 and inhibited S100A8-mediated activation, but enhanced cytokine induction by LPS. In contrast, TLR4 is implicated in monocyte and DC activation by S100A9. Another study showed a requirement for Ca2+ and Zn2+ to achieve high-affinity S100A9 binding to TLR4/MD2, whereas S100A8/A9 binding was less. S100A12 is also recently reported to activate human monocytes via TLR4 rather than RAGE.

In contrast to these results, we have been unable to activate human or murine monocytes/macrophages or EC with any calgranulin. Our preparations were validated for purity and structural integrity, and lacked endotoxin, and were tested at various concentrations using stringent LPS-free culture conditions.
Functions of S100 Proteins in Atherogenesis

ic inflammation in mice should be interpreted with caution, given the differences in expression patterns of key mediators in humans and mice.

S100A8 is several hundred-fold more susceptible to HOCl oxidation than LDL. Until recently, the ROS-scavenging capacities of S100A8 and S100A9 in human disease were unproven, although cross-linked forms in diseased carotid arteries, reactive with an antibody raised to HOCl-oxidized S100A8, implicated this process in atherogenesis. However, isolation of these components from carotid samples proved difficult because these modifications are not detected using conventional in-gel protocols. Using purified human asthmatic sputum as an alternate inflammatory source, we found oxidation products identical to those generated by HOCl in recombinant S100A8; sulfinic and sulfonic acid intermediates, and novel oxathiazolidine oxide/dioxide adducts on Cys42 were confirmed and Met 1 and Met 78 and Trp 54 were oxidized, strongly supporting the notion that S100A8 contributes to antioxidant defense in human inflammatory processes.

S100A9 has weaker oxidant scavenging capacity than S100A8, but ROS may promote structural changes that alter its fugetactic activity. Unlike S100A8, S100A9 is readily S-glutathionylated, and this product reduces neutrophil adhesion to fibronectin, and their binding affinity to EC. However, 2 forms of S100A9 are expressed; the truncated form lacking the single Cys3 residue comprises approximately one-third of the total S100A9 in neutrophils, but more research is required to characterize this in inflammatory lesions. However, in contrast to earlier reports, we found that neither native nor modified S100A8 and/or S100A9 changed EC adhesion molecule or cytokine expression.

S100A8 Scavenges Oxidants S100A8, an efficient 2-electron scavenger, may modulate the redox balance at inflammatory sites. In addition to its rapid oxidation by peroxide, novel oxidative modifications of the single reactive Cys42 residue in murine S100A8 are generated by hypohalous acid oxidants. Functional implications were recently reviewed, and include modulation of its chemotactic and pro-adhesive properties. Phagocyte activation promotes MPO-driven HOCl generation that contributes to vascular dysfunction and oxidative damage in atherosclerosis, generally where S100A8 and S100A9 are plentiful. Interestingly, murine macrophages produce little MPO, and this may explain the lack of S100A8 in the atheroma of mice. Thus, extrapolation of results of studies of chronic inflammation in mice should be interpreted with caution, given the differences in expression patterns of key mediators in humans and mice.

S100A8 is several hundred-fold more susceptible to HOCl oxidation than LDL. Until recently, the ROS-scavenging capacities of S100A8 and S100A9 in human disease were unproven, although cross-linked forms in diseased carotid arteries, reactive with an antibody raised to HOCl-oxidized S100A8, implicated this process in atherogenesis. However, isolation of these components from carotid samples proved difficult because these modifications are not detected using conventional in-gel protocols. Using purified human asthmatic sputum as an alternate inflammatory source, we found oxidation products identical to those generated by HOCl in recombinant S100A8; sulfinic and sulfonic acid intermediates, and novel oxathiazolidine oxide/dioxide adducts on Cys42 were confirmed and Met 1 and Met 78 and Trp 54 were oxidized, strongly supporting the notion that S100A8 contributes to antioxidant defense in human inflammatory processes. Figure 3 emphasizes the functions of S100A8 that are involved in redox-mediated reactions.

S100A9 affects DC maturation and function and so may modulate acquired T cell immunity. For example, S100A9 deficiency worsens cardiac allograft survival, but unlike models of atherogenesis, is associated with increased leukocyte accumulation and expression of IFN-γ and IFN-γ-inducible chemokines. S100A9 was proposed to regulate the antigen-presenting capacity of DCs by modulating co-stimulatory molecule expression in this model. Similarly, S100A9-/- mice have more severe allergic contact dermatitis. In this case, S100A8 inhibited early DC maturation and antigen presentation in a TLR4-dependent manner.
Effects of Calgranulins on MC Function  Murine models confirm MC involvement in atherosclerotic plaque progression and destabilization, and in abdominal aortic aneurysms (reviewed in Bot & Biessen\textsuperscript{96} and Wang & Shi\textsuperscript{97}). Connective tissue MC are abundant in diseased arteries and tryptase and chymase released following MC activation may contribute to extracellular matrix destruction.\textsuperscript{98} Preformed mediators released from granules include TNF, interleukins, histamine and lipid.
mediators, and genes encoding chemokines and factors that influence specific lymphocyte populations and facilitate leukocyte recruitment into lesions. Figure 4 summarizes some likely functions of MC in atherosclerosis.

Endogenous factors, other than classical IgE-mediated activation, that influence MC function are still being identified. The calgranulins have pleiotropic effects that may influence atherosclerosis. We showed that S100A12 is a MC chemotactant in vitro and sequesters MC in vivo, and promotes degranulation and histamine release that facilitates vasculature changes conducive to leukocyte recruitment. In particular, S100A12 increased the flux of rolling leukocytes in the rat mesenteric circulation, with concomitant increases in adherent leukocytes. S100A12 induced chemokines (MCP-1, IL-8, MIP-1β) and IL-6 and TNF-α, whereas IgE-cross-linking generated additional factors important in allergic inflammation.31

Unexpectedly, transgenic mice overexpressing S100A12 in SMC had attenuated symptoms of acute murine asthma. RAGE and TLR4 are highly expressed in the lung, and because S100A12 was reported to activate these, amplification of inflammation was predicted.105 In this model, eosinophil infiltration and perivascular inflammation were suppressed because of reduced chemokine production. SMC abnormalities that suppressed airway responsiveness may have been due to reduced inflammation caused by myocyte apoptosis.106 One caveat of this study is that S100A12 expression is only obvious in macrophages and eosinophils, but not in SMC in the human asthmatic lung,144 and its overexpression in murine SMC may be unphysiological.

In contrast to S100A12, S100A8 inhibits MC activation, at least partially by scavenging ROS required for signaling. Possible counterexamples of S100A12 and S100A8 on mast cell activation are shown in Figure 5. Intranasal S100A8 administration reduced MC activation, generation of chemokotransmitters, and symptoms of acute murine asthma.107 Additionally, S100A8-SNO from neutrophils increased following treatment with NO donors; S100A9 was not modified. Only a few proteins have the signature sequence required for S-nitrosylation108 and its overexpression in murine SMC may be unphysiological.

S100A12 Blunts Proinflammatory Cytokine Production Induced by Serum Amyloid A

SAA is an acute-phase reactant elevated in serum from CVD patients109 and SAA enrichment of high-density lipoprotein reduces its anti-inflammatory properties.110 In human atheroma, SAA is detected in EC, SMC, foam cells and adventitial macrophages,111 suggesting a localized role. Its overexpression in ApoE−/− mice increased plasma proinflammatory cytokines and accelerated atherosclerosis.112 SAA has proinflammatory and prothrombotic activities in vitro, promoting cytokine production in neutrophils,113 monocytes,114,115 lymphocytes,116 and EC,117 and is a leukocyte chemotactant.118,119 SAA is also a potent inducer of tissue factor in human monocytes120 and EC,121 indicating prothrombotic properties, and causes endothelial dysfunction by enhancing ROS production and downregulating endothelial NOS (eNOS).119,120

Low S100A12 concentrations (50 nmol/L), suppressed SAA-, but not LPS-induced cytokine production from human peripheral blood mononuclear cells, whereas S100A8 or S100A9 were ineffective. Suppression was not via IL-10 induction, or effects on NF-κB-mediated signaling, but likely via the ERK1/2 MAPK pathway. Interestingly, S100A12 did not affect monocyte tissue factor induced by SAA, indicating divergent pathways upstream of ERK1/2.52 We propose that localized S100A12 may modulate sterile inflammation to blunt the pro-inflammatory properties of lipid-poor SAA deposited in atherosclerotic lesions, where both are elevated as a consequence of macrophage activation.

Conclusions

Clinical studies implicate raised circulating levels of calgranulins in the pathogenesis of CVD, principally because of their associations with proinflammatory processes. Atherosclerosis is a chronic disease with potentially fatal consequences, but mechanisms controlling the magnitude of inflammation are poorly understood. We propose that the abundance, and types of cells expressing calgranulins in particular microenvironments, their relative concentrations and post-translational modifications may have distinct functional outcomes, including those that are protective at different stages of atherogenesis. For example, very low calgranulin levels may promote directed neutrophil migration in the initial phases. Following neutrophil and monocyte/macrophage accumulation and activation in plaque, the high concentrations of S100A8 released may moderate oxidative damage and reduce inflammation by suppressing MC activation. In addition, localized induction of cytokines by SAA deposits may be blunted by S100A12,52 and T cell proliferation reduced by S100A8/A9.53 S100A8-SNO from neutrophils and neovessels in atheroma may contribute to vessel homeostasis. These mechanisms provide new perspectives into the regulatory functions of the calgranulins that could potentially limit atherogenesis.

Disclosures

Grants: Research funded by grants #630647 and #1027189 from the National Health and Medical Research Council of Australia.

References

link between inflammation and oxidative stress in hyperlipidemic patients. *Atherosclerosis* 2012; 220: 372–379.


33. Kondörfer IP, Brueckner F, Skerra A. The crystal structure of the human (S100A12/S100A9)2 heterotetramer, calprotectin, illustrates how conformational changes of interacting a-helices can determine specific association of two EF-hand proteins. *J Mol Biol* 2007; 370: 877–898.


81. Hofmann Bowman MA, Schmidt AM. S100/calgranulins EN-RAGE the blood vessels: Implications for inflammatory responses.


