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Emerging roles of neutrophil-borne S100A8/A9 in cardiovascular inflammation.

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Abstract

Elevated neutrophil count is associated with higher risk of major adverse cardiac events including myocardial infarction and early development of heart failure. Neutrophils contribute to cardiac damage through a number of mechanisms, including attraction of other immune cells and release of inflammatory mediators. Recently, a number of independent studies have reported a causal role for neutrophil-derived alarmins (i.e. S100A8/A9) in inducing inflammation and cardiac injury following myocardial infarction (MI). Furthermore, a positive correlation between serum S100A8/A9 levels and major adverse cardiac events (MACE) in MI patients was also observed implying that targeting neutrophils or their inflammatory cargo could be beneficial in reducing heart failure. However, contradictory to this idea, neutrophils and neutrophil-derived S100A8/A9 also seem to play a vital role in the resolution of inflammation. Thus, a better understanding of how neutrophils balance these seemingly contrasting functions would allow us to develop effective therapies that preserve the inflammation-resolving function while restricting the damage caused by inflammation. In this review, we specifically discuss the mechanisms behind neutrophil-derived S100A8/A9 in promoting inflammation and resolution in the context of MI. We also provide a perspective on how neutrophils could be potentially targeted to ameliorate cardiac inflammation and the ensuing damage.

Keywords

DAMPs; Cardiac Inflammation; S100A8/A9; Neutrophils; Myocardial Infarction

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Introduction

Acute coronary syndrome (ACS) is one of the leading causes of death in the modern world. Advances in coronary care and early reperfusion strategies have dramatically reduced the mortality rates but has also resulted in a large pool of subjects who are at risk of developing heart failure or who have developed heart failure and require heart transplants(1). Development of heart failure following myocardial infarction (MI) is influenced by the size of the infarct and the inflammatory response it elicits. The efficiency of cardiac reparatory mechanisms also dictate the outcome of MI(2). Cardiac repair is dependent on a well-coordinated inflammatory response that begins with the recruitment of neutrophils (within hours) followed by monocytes (1-3 days), macrophages and adaptive immune cells(3). An increase in the severity or prolongation of the immune response may result in adverse remodeling and heart failure (4, 5). It was originally hypothesized that attenuating of inflammation would diminish the recurrence of ACS but indiscriminate targeting of inflammation has been shown to be counter-productive. In fact, use of drugs with broad anti-inflammatory qualities such as steroid/non-steroid anti-inflammatory agents is not recommended in the early stages of MI (6). More targeted strategies like inhibition of interleukin (IL-1 β) or disruption of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activity may achieve better outcomes following a MI (7, 8). The recently concluded Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) using a human monoclonal antibody against IL-1 β in patients with previous MI and elevated high-sensitivity C-reactive protein (hsCRP) demonstrated a much better clinical success. Canakinumab treatment reduced the relative risk of non-fatal MI, non-fatal stroke, or cardiovascular death by 15% compared with placebo although the overall mortality did not differ significantly between the various groups(9, 10). Another trial, The Colchicine Cardiovascular Outcomes Trial (COLCOT) that investigated the efficacy of colchicine to reduce the risk of cardiovascular events after MI found similar data (11). Colchicine administration to MI patients lowered the risk of stroke and urgent hospitalization for angina leading to coronary revascularization by 23%(12). Colchicine is an anti-inflammatory agent that inhibits microtubule polymerization to affect cell mitosis and neutrophil motility (13). Colchicine also inhibits NLRP3 inflammasome activation and reduces the levels of IL-6 and CRP (13, 14). These drug trials reiterate the concept that pharmacological strategies aimed at selective inhibition of inflammatory molecules such as IL-1 β is a better approach in managing cardiovascular disease.

Increased levels of IL-1 β can induce myelopoiesis and myeloid cells can be activated to stimulate further IL-1 β release, thus creating a feedforward loop producing more leukocytes and inflammatory cytokine(15–17). The benefits accrued from blocking IL-1 β signaling in the CANTOS and COLCOT trials may in part be derived from suppression of leukocytosis(9). Elevated WBC count especially the neutrophil count is a marker of inflammation and is associated with high risk of adverse clinical outcomes(18) including myocardial infarction and early development of congestive heart failure(19, 20). Neutrophil count is also considered an independent prognostic factor in patients with ACS both on admission and after revascularization by percutaneous coronary intervention (PCI) or coronary bypass surgery(21). Further, elevated neutrophil count is directly related to infarct

size and decline in left ventricular ejection fraction (LVEF)(22) suggesting that neutrophil count post-procedurally is an independent predictor of cardiovascular outcomes. Mirroring the efforts at broad suppression of inflammation, strategies aimed at reducing neutrophil recruitment/activation have been shown to limit tissue injury but the translation of this therapeutic potential to the clinical arena has not been successful(23, 24). Much like inflammation, neutrophils have also been shown to activate reparative responses after MI(25). A precise understanding of the effectors used by neutrophils to balance its inflammatory/ reparative functions would allow us to take a more directed approach in developing effective therapies. These novel therapies should allow us to preserve the inflammation-resolving function of neutrophils while restricting the damage caused by an inflammatory overkill. Emerging evidence suggest that the neutrophil-derived alarmins such as S100 calcium binding proteins (S100A8 and S100A9) play a major role in determining the nature and magnitude of inflammatory response post-MI(26–28). Interestingly, these proteins also seem to play a role in the resolution of inflammation(29). S100A8/A9 closely mirror the role of neutrophils both in inflammation and reparative response and, may in fact be the main effector molecules used by neutrophils to manage these contrasting functions. Thus, a clear understanding of the role of S100A8/A9 including neutrophil kinetics and the signaling mechanisms that regulate S100A8/A9 release from neutrophils is vital for developing novel neutrophil-targeted therapeutics. In the following sections, we will discuss how the multifaceted functions of S100A8/A9 play a role in inflammation and resolution phases of ACS. We will also discuss the role of S100A8/A9 in myelopoiesis and induction of cardiomyocyte death and novel strategies to tone down inflammation.

Physiological regulation of neutrophil production

Neutrophils are produced by a process termed as granulopoiesis. It is a well-orchestrated *de novo* generation that results from increased proliferation of myeloid precursors primarily in the bone marrow (BM) as well as spleen. In times of inflammatory injury an innate response is to increase production of neutrophils, which can be defined in two ways. ‘Emergency granulopoiesis’ describe a microbial infection-driven process, while ‘reactive granulopoiesis’ describes a form of enhanced granulopoiesis caused by physical or chemical insults(30). Neutrophils are produced within hematopoietic cords of the BM, regulated by selective expression and coordinated interplay between key transcription factors including PU.1, CCAAT/enhancer-binding proteins (C/EBP), etc.(31, 32). In a steady state, granulopoiesis is stimulated by granulocyte colony stimulating factor (G-CSF)(33), a growth factor that is mainly produced in immune cells including neutrophils by exerting its effects on G-CSF receptor(33). G-CSF has a wide range of effects from forcing the hematopoietic multipotential stem and progenitor cells (HSPCs) towards myeloid lineage (via enhanced expression of PU.1 and C/EBP β)(34, 35) proliferation of granulocyte precursors, reduction in transit time through the granulocytic compartment(36), and release of mature neutrophils from the BM (*via* the suppression of CXC-chemokine receptor 4 (CXCR4)-stromal derived factor (SDF1) axis)(37) (38),(39). Once produced, neutrophils usually reside in the BM for 6-7 days, but could be expedited during emergency situations and the forced out immature cells appear as “left shift” in peripheral smears. The overall post-mitotic transit time in humans is estimated to be around about 4-6 days with a daily turnover of 5 to 10¹⁰ cells per

day (40, 41) and nearly 90% of them remain in the BM(38). Because of relatively short half-life, the neutrophil pool has to be constantly replenished through continuous proliferation of hematopoietic stem cells (HSC), common myeloid (CMP) and granulocyte-macrophage progenitor (GMP) cells. Once released, the mature neutrophils distribute themselves almost equally between two compartments, circulating and marginal(40). These two pools remain in a dynamic equilibrium but could be disturbed by innocuous stimuli such as exercise, physiological stress or a surge in glucocorticoids (40). The mechanisms that promotes demargination are unclear but likely involves shedding of L-selectin (CD62L)(42) on the surface of neutrophils by Adam17, a cysteine metalloproteinase(43, 44). The distribution of neutrophils within the system also varies and depends on the maturation and activation status. For example, mature peripheral blood neutrophils localize mainly to the liver while the younger cells could return to their home in BM(45). Neutrophil homeostasis is maintained by a delicate balance between granulopoiesis, BM storage and release, intravascular margination, clearance and destruction(38).

Neutrophilia during myocardial infarction

In response to ischemic injury, the number of neutrophils in the blood increases rapidly and begin to appear in the infarct within hours(46). The precise mechanism that increase the number of circulating neutrophils post-MI is unclear. Neutrophils are normally retained in the BM by SDF-1, a retention factor, through its interaction with CXCR4 and any disruption to this axis could enhance rapid egress of neutrophils(47). However, acute mobilization of neutrophils from the BM might require a well-coordinated interplay between G-CSF and CXC chemokines, with G-CSF disrupting the CXCR4-SDF-1 axis and CXC chemokines provide a chemical gradient for neutrophil chemotaxis across the BM endothelium(48). Previous studies have shown that disruption of CXCR4-SDF1 axis promotes mobilization of HSPCs from the BM to spleen to enhance extramedullary leukocytosis(49). Activation of sympathetic nervous system (SNS) or increase in NE levels post-MI could also induce neutrophilia *via* disruption of CXCR4-SDF1 axis(50) suggesting that stress could play a role in MI-induced granulopoiesis. However, to what extent SNS-induced stress play a role in sustained granulopoiesis once the NE levels subside is unclear. Studies have shown that SNS signaling along with local signals from the infarct may stimulate the release of HSCs from the BM, which then migrate to spleen to promote extramedullary hematopoiesis(49). Thus, both medullary and extramedullary granulopoiesis may contribute to the overall circulatory pool of neutrophils in response to MI.

Granulopoiesis during myocardial infarction: Role of DAMPs

As stated earlier, in a steady state condition, production of neutrophils is tightly regulated by G-CSF, a cytokine mainly produced from immune cells including neutrophils via transcription regulation of PU.1 and C/EBP α /e in myeloid precursors in the BM(34, 51). However, in conditions of sterile injury such as MI, granulopoiesis could be initiated by the damage-associated molecular patterns (DAMPs) and other cytokines (e.g. IL-6, IL-3, GM-CSF etc.) resulting in heightened production of neutrophils from the BM(30, 52). Recent studies have suggested that soluble factors (e.g. IL-1 β , S100A8/A9) released from necrotic cells have the ability to promote granulopoiesis by interacting with their receptors on

myeloid cells in the BM (7, 15, 53). Among the different cardiac cells, leukocytes are the major contributors of soluble factors. Global transcriptome profiling of different cell types in the infarct including cardiomyocytes, fibroblasts, endothelial cells and leukocytes has revealed hierarchical activation of inflammatory response mainly in CD45 cells highlighting the role of leukocytes in shaping up the inflammatory response (28, 54). Within this diverse population of leukocytes, neutrophils dominate the cellular landscape and contribute significantly to the local and systemic pool of chemokines, cytokines and DAMPs(28). Previous studies have reported increased expression of various chemokines (e.g. Cxcl1, Cxcl2, Ccl3, Cxcl5, CCL2 etc.) and inflammatory cytokines (TNF α , IL6, etc.) post-MI (46) but most dramatic was the expression of S100A8 and S100A9 proteins (28). Further studies utilizing cell-sorting techniques combined with qRT-PCR and confocal imaging identified neutrophils as the predominant source of S100A8/A9 in the infarct(28). In the following sections, we will describe the basic biology of S100A8/A9, their myriad intracellular and extracellular functions including the recently discovered role in MI-induced granulopoiesis and ischemic injury.

S100 calcium-binding proteins (S100A8/A9), more than just DAMPs.

S100A8 and S100A9 (a.k.a myeloid-related protein, MRP-8 and MRP-14 respectively) are endogenous DAMPs/alarmins belonging to the S100 family, which are located as a cluster on chromosome 1q21(55). They are constitutively expressed in myeloid cells and are stored as granules-ready to be released in response to inflammatory/infectious stimuli (56). They usually exist as homodimers but preferentially form heterodimers which is necessary for their biological effects (57). The secretory mechanisms of S100A8/A9 have also been elusive, partly due to the lack of an identifiable leader sequence that would facilitate its secretion via the classical endoplasmic reticulum-Golgi pathway. The general consensus is that secretion of S100A8/A9 is dependent on the production of ROS and may require K⁺ exchange through the ATP-sensitive K⁺ channels (58). Emerging evidence suggest that S100A8/A9 is released during NETosis (59), a form of cell death that involves extrusion of decondensed chromatin and granular contents to the extracellular space. Interestingly, S100A8/A9 forms the bulk of NET bound cytosolic proteins (60). In the setting of MI, the ischemic microenvironment (with excess ROS, Ca²⁺, etc.) is conducive for both activation of neutrophils and cell death by NETosis. It is likely that NETosis is essential for S100A8/A9 release in the heart post-MI (61).

Several other DAMPs such as high mobility group box-1 (HMGB1), ATP, uric acid, fibronectin extra domain A, interleukin(IL)-1 α , heat shock proteins (HSPs), low molecular weight hyaluronic acid, complement, mitochondrial DNA, dsRNA and ssRNA are also implicated in mediating the inflammatory response post-MI (62). They bind to either toll-like receptor/IL-1 receptors (TLR/IL-1Rs), cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and receptor for advanced glycation end-products (RAGE) and activate mitogen-activated protein kinases (MAPKs) and nuclear factor (NR- κ B) pathways to ultimately activate the inflammatory cascade (62). Unlike other DAMPs, S100A8/A9 play a major role in the activation of inflammatory response after MI, as they are highly expressed in neutrophils, (constituting as much as 45% of all cytosolic proteins) and neutrophils dominate the early immune response post-MI (28). Interestingly, the initial

wave of infiltrating neutrophils seems to set the pace for amplification of inflammation by promoting large-scale release of S100A8/A9. The released S100A8/A9 acting either in an autocrine or paracrine fashion interact with TLR4 on naïve neutrophils, prime the Nlrp3 inflammasome and promote secretion of IL-1 β . The released IL-1 β travel to BM, interact with its receptor (i.e. IL1R1) on hematopoietic stem and myeloid progenitor cells in the BM and stimulate myelopoiesis in a cell-autonomous manner(63). Various genetic and pharmacological strategies aimed at disruption of the S100A8/A9-Nlrp3-IL-1 β signaling axis have been shown to dampen myelopoiesis and, improve cardiac function (17, 63). These findings suggest that S100A8/A9 act locally within the ischemic environment to help sustain the inflammatory response by promoting IL-1 β release from the primed neutrophils. It could be concluded that during MI and probably other ischemic injuries, neutrophils self-regulate their own and other leukocyte production by deploying S100A8/A9.

In addition to alerting the innate immune system by triggering leukocytosis, S100A8/A9 could also induce a direct myocardial injury during MI. In a study published by Li *et al* (26), it was found that S100A8/A9 has direct detrimental effects on mitochondrial function in cardiomyocytes. Using dynamic transcriptome analyses of mouse hearts exposed to various durations of ischemic and reperfusion injuries, Li *et al* found that S100A8/A9 released from infiltrating neutrophils inflicted irreversible damage to the mitochondrial electron transport chain complex 1 (ETC1) causing cardiomyocyte death by apoptosis. They found that in response to CXCL1, a specific chemokine released from necrotic cardiac cells, neutrophils were rapidly recruited to the ischemic myocardium with the aid of CXCR2, a chemotactic receptor that is essential for neutrophils migration. At the infarct, cardiomyocyte cell death was induced by S100A8/A9 binding to TLR4 and stimulating a cascade of events leading to functional defects in mitochondrial ATP bioenergetics, mitochondrial membrane potential and ATP synthesis. These damages were mitigated in mice that lacked S100A8/A9, while further exacerbated in transgenic mice that overexpressed S100A8/A9. Further, all the above findings were robustly recapitulated *in vitro* using isolated cardiomyocytes subjected to hypoxia and stimulation by S100A8/A9. These data confirmed a direct role for neutrophil-derived S100A8/A9 in inflicting a severe injury by suffocating cardiomyocytes to apoptotic death.

Another feature that distinguish S100A8/A9 from other DAMPs is the multiplicity of intracellular and extracellular functions that it displays. The intracellular role of S100A8/A9 involves regulation of microtubule reorganization (64), arachidonic acid (AA) metabolism and trafficking (65, 66), calcium-dependent signaling (67, 68) and integration of mitogen-activated protein kinase activity (64). On the other hand, the extracellular role of S100A8/A9 is facilitated by binding to TLR-4 (69), RAGE (67), CD36 (70) and possibly other receptors (71). In the following sections, we will elaborate the role of S100A8/A9 in the pathology of vascular and cardiac inflammation.

The myriad functions of S100A8/A9 in cardiovascular disease:

1. Vascular inflammation

By virtue of its multi-faceted functions (55), S100A8/A9 seems to be involved in many steps of the inflammatory process, beginning from the initiation to resolution of inflammation

(Summarized in Table 1). For example, S100A9 is necessary for accumulation of neutrophils and macrophages in the inflamed tissue across multiple disease models of vascular inflammation (72). Extracellular S100A8/A9 can function as a chemoattractant by binding to receptors on leukocytes and activating them (67, 69, 73, 74), while also inducing phagocyte migration (75). Cytokine and chemokine activation of Rac1 and Cdc42 to induce cell migration is dependent upon antagonizing the stabilizing effect S100A8/A9 on microtubules (64). S100A8/A9 can also promote the accumulation of leukocytes at the sites of inflammation by regulating myeloid adhesion and transmigration across the blood vessel wall (65, 76–80). Increased serum levels of S100A8/A9 can induce CD11b on circulating monocytes, leading to accumulation of monocyte-derived macrophages and dendritic cells (DCs) in pancreatic islets (81). S100A8/A9 can induce expression of vascular cell adhesion molecules (VCAM-1, ICAM-1) on endothelial cells to enhance trans-endothelial migration of leukocytes (82). S100A8/A9 binding to TLR4 and RAGE activates inflammatory pathways that lead to secretion of multiple cytokines that sustain or exacerbate inflammation (69, 83, 84). S100A8/A9 also promotes proliferation of inflammatory leukocytes via differentiation of myeloid progenitor cells in the bone marrow (BM) and increase the production of inflammatory Ly6C^{hi} monocytes and neutrophils in the atherosclerotic lesions (53).

Intracellular functions of S100A8/A9 also play a role in vascular inflammation. *S100a9*^{−/−} neutrophils have reduced Mac-1 expression at baseline and in response to cell activation (76) and S100A8/A9 may regulate leukocyte adhesion during vascular inflammation through control of Mac-1 (76, 78, 85). S100A9-deficient neutrophils exhibit a reduced secretion of cytokines (86). S100A8/A9 binding to arachidonic acid (AA) results in activation of NADPH oxidase (NOX) which is involved in reactive oxygen species (ROS) production during inflammation (87–91). S100A9 is expressed in atherosclerotic lesions (92) and increased presence of S100A8/A9 positive macrophages is associated with rupture prone lesions (93). Studies in mouse atherosclerotic models have found an increased expression of S100A8/A9 in monocytes and activated macrophages that infiltrate atherosclerotic lesions and are responsible for plaque instability and rupture (94, 95). S100A8 expression is also reduced in macrophages of regressing lesions (96). Increased accumulation of S100A9 is also seen in monocytes and neutrophils that accelerates atherosclerotic lesion disruption in diabetic mice (86, 97, 98). Non-myeloid cell-derived S100A8/A9 may also impair endothelial integrity (99), induce chemotactic factor expression in endothelial cells (100), and promote VSMC proliferation (101).

2. Cardiac inflammation

Following an MI, many S100 family members are involved in activation of pro-inflammatory cascades in the infarcted heart (102–104). S100A8/A9 secreted from activated myeloid cells in the infarct binds to TLR4 and RAGE of various cell types, activating NF- κ B and mitogen-activated protein kinase (MAPK) pathways (105, 106). The activation of these signaling cascades leads to recruitment of more inflammatory cells (107), induction of inflammatory cytokines and chemokines (16) and an increased synthesis of proteases, and extracellular matrix (ECM) proteins (108). S100A8/A9 binding to RAGE on cardiomyocytes in sepsis can lead to cardiomyocyte dysfunction due to decrease in cardiomyocyte

contractility caused by reduced calcium flux (67). Intracellular S100A8/A9 can induce oxidative bursts in myeloid cells and further amplify inflammation by transferring AA to the NADPH oxidase complex (109).

Plasma levels of S100A8/A9 is considered as a marker of cardiovascular risk in humans(110). S100A9 expression increases before STEMI, and S100A8/A9 plasma levels in healthy individuals are predictive of the risk of future cardiovascular events (111). S100A8/A9 levels are elevated in systemic circulation prior to other markers of myocardial necrosis and may represent a mediator and early biomarker of ACS (112). In the Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial, patients with cardiovascular death or MI after 30 days had higher median S100A8/A9 levels than patients who remained free of recurrent events. Thus, elevated levels of S100A8/A9 showed significantly increased risk of cardiovascular death or MI (113).

The anti-inflammatory effects of S100A8/A9

The S100 family members are not only mediators of inflammation but can also be anti-inflammatory and have a specific role in resolving inflammation (114–116). Functions of S100 proteins are very much context specific with interactions between other members of S100 family, intracellular binding partners as well as post-translational modifications such as oxidation and phosphorylation events playing a role in deciding its function. For example, S100A9 deficiency in neutrophils leads to reduced secretion of cytokines in response to TLR-4 activation but in dendritic cells it leads to an exacerbated release of cytokines (117). For all its role in activation and aggravation of inflammation, S100A8/A9 also has a counter role in controlling runaway inflammation (118–128). S100A8/A9 can repel leukocytes from the sites of inflammation (129, 130) and deactivate macrophages (131). In addition to the oxidation enhancing effect of S100A8/A9, a ROS scavenging effect has also been reported for S100A8 (132, 133). S100A8/A9 can also suppress NF- κ B activation to reduce mRNA expression of various inflammatory molecules such as IL-1 β , IL-6, and TNF- α in the myocardium (134). S100A8/A9 can also dampen inflammatory response by binding to and mopping up inflammatory cytokines IL-1 β , IL-6, and TNF- α in vivo but the molecular mechanisms remain unclear (134).

The mechanisms behind the disparate functions of S100A8/A9 are unclear but it is likely that a particular oligomeric form or post-translational modification may define their function(135). Furthermore, different sub-sets of neutrophils (matured vs immature) mobilized to the heart may secrete distinct forms of S100A8/A9 (e.g. phosphorylated S100A9)(59), or a common form secreted by neutrophils may show functional plasticity depending on the calcium levels in the extracellular milieu. S100A8/A9 may display a pro-inflammatory or reparative role depending on the cellular context and stage of inflammation. Preexisting conditions like autoimmune diseases or diabetes mellitus may also influence the ratio of neutrophil sub-sets or the extracellular milieu encountered by S100A8/A9 and, thus may impact S100A8/A9 function. Thus, the functions of S100A8/A9 are very much context-specific and identification of the right window of opportunity for intervention is key to developing novel therapies aimed at reducing the disease burden(136).

Targeting S100A8/A9 during MI: In search of a window of opportunity

S100A8/A9 has both pro- and anti-inflammatory functions in the context of a CVD but the precise molecular mechanism involved in its anti-inflammatory effects were unknown until recently. In an elegant study by Marinkovic *et al*, it was demonstrated that short-term pharmacological inhibition of S100A8/A9 with a small molecule inhibitor (that prevents S100A8/A9 binding to its receptors, TLR4 and RAGE) mitigated both local and systemic inflammation, and as expected reduced myocardial damage after MI (27). Interestingly, long-term inhibition of S100A8/A9 with the same drug led to deterioration of cardiac function over time(29). Examining the reasons for such an anomaly, it was later identified that extended inhibition of S100A8/A9 beyond the inflammatory phase (> 3 days), resulted in decreased proliferation of stem cells in the BM. The deficiency of these cells which are important for monocytoesis led to a reduction in the recruitment of anti-inflammatory monocytes to the myocardium and their subsequent conversion to reparative macrophages. The delay in this conversion may have led to defective clearance of apoptotic cardiomyocytes and thus, resulted in a larger myocardial scar. Mechanistically, it was found that S100A8/A9 is directly involved in the maturation of Ly6C^{hi} monocytes to Ly6C^{lo} reparative macrophages through induction of the nuclear receptor subfamily 4 group A member 1 (Nr4a1) expression. It is important to recognize that Nr4a1 is critical for upregulation of Mertk, the phagocytosis receptor (137, 138) required for efficient clearance of debris and dead cells (5, 137, 139). Taken together, these findings confirm that neutrophil-derived S100A8/A9 is indeed a double-edged sword during MI. On one hand, S100A8/A9 interacts with TLR4 on naïve neutrophils to amplify the inflammatory response and induce cardiomyocyte apoptosis. On the other hand, S100A8/A9 aid in the conversion of Ly6C^{hi} cells to Ly6C^{lo} reparative macrophages that is vital for effective clearance of dead cells/debris (Schematic Figure).

Compounds like ABR-238901, tasquinimod (ABR-215050) and paquinimod (ABR-215757) have been shown to block the interaction of S100A8/A9 with TLR4 and RAGE (21, 29, 140, 141). These compounds have been reported to reduce inflammation and disease progression in a number of inflammatory models (142–146). Tasquinimod is in phase 3 clinical trial for treatment of prostate cancer (147) and if approved, could be potentially repurposed to target cardiac inflammation post-MI. Interventions to reduce S100A8/A9 secretion by inhibiting NETosis may also have suppressive effect on inflammasome activation and subsequent secretion of IL-1 β (61). Another strategy to consider is pre-emptive inhibition of NLRP3 inflammasome(148) or Gadermin D, a protein that is critical for IL-1 β release (149), to forestall the deleterious effects of IL-1 β . Encouraging results from animal studies have demonstrated that reduction in markers of NLRP3 activation (e.g., IL-1 β) correlate with improvement in plague progression suggesting that if initiated early enough, such inhibitors could maintain atherosclerosis at a sub-clinical level(150). Furthermore, a number of small molecule NLRP3 inhibitors (direct and indirect) are reported to reduce damage to the heart muscle post-MI suggesting a promising potential for treatment of heart failure (8, 151, 152). Some of these inhibitors have progressed to clinical trials and are at different stages of development. However, none of them is approved to treat CVD yet.

Summary and conclusions

In summary, neutrophil-derived S100A8/A9 presents itself as a viable and feasible target to achieve better clinical success in ACS patients. Because S100A8/A9 is involved in activation of both the inflammatory and reparative phases of MI, blockade of S100A8/A9 at the right therapeutic window might represent a feasible strategy to improve prognosis in ACS patients. However, it is not an easy task to identify the so-called “therapeutic window” in patients who presents injuries of varying magnitude, inflammatory response and co-morbidities. Furthermore, there are no accurate and reliable diagnostic markers yet to the define the boundary between the pro-inflammatory and anti-inflammatory phases of S100A8/A9 actions. We propose that a more moderate and short-term inhibition of S100A8/A9 during the acute inflammatory phase (1-3 days post-MI) particularly at the time of admission or immediately after PCI may be most beneficial in the setting of acute MI. Furthermore, monitoring neutrophil count in the blood or plasma levels of S100A8/A9 post-procedurally may help validate neutrophils and S100A8/A9 as diagnostic markers in a clinical setting.

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schematic figure. Proposed model showing how neutrophil derived-S100A8/A9 perpetuates inflammation, myocardial injury and resolution of inflammation:

Following myocardial infarction (MI), neutrophils are rapidly recruited to the ischemic heart (via an interaction between CXCR2 and CXCL1) where they release S100A8/A9. The released S100A8/A9 bind to TLR4 on naïve neutrophils and prime the Nlrp3 inflammasome and release of IL-1 β which in turn interact with its receptor (IL1R1) on myeloid progenitor cells in the bone marrow to stimulate myelopoiesis³. On the other hand, S100A8/A9 interact with TLR4 on cardiomyocytes and induce apoptotic death by repressing NDUF genes that are essential for ETC complex I formation in mitochondria. Suppression of ETC complex I activity is likely mediated by downregulation of NRF1 and PGC1 α leading to mitochondrial dysfunction and ultimately cell death. Short-term treatment with a small molecule chemical inhibitor (ABR-238901) ameliorates myocardial injury by reducing S100A8/A9-induced inflammation. Long-term blockade leads to progressive decline of cardiac function and left ventricle dilatation by suppressing S100A8/A9-mediated induction of Nur77, a transcription factor that is essential for maturation of monocytes to reparative monocytes and macrophages.

NDUF, NADH dehydrogenase (ubiquinone) Family; NRF1, nuclear respiratory factor 1; PGC-1 α , Pparg coactivator 1 alpha; ETC, Electron Transport Chain; HSC, Hematopoietic Stem Cell; CMP, Common Myeloid Progenitor; GMP, Granulocyte Macrophage Progenitor and TLR4, Toll-like receptor 4.

Table 1:

Summary of S100A8/A9 functions in cardiovascular disease

Intracellular functions	Microtubule reorganization	(64)
	Arachidonic acid metabolism and trafficking	(65, 66)
	Calcium-dependent signaling	(67, 68)
	Integration of MAP kinase activity	(64)
	ROS production	(87–91)
	Cytokine production	(69, 83, 84, 86)
Extra-cellular functions	Activation of inflammation	(67, 69–71, 83, 84)
	Accumulation of immune cells in tissue	(64, 65, 67, 69, 72–81, 85)
	Endothelial cell activation	(82, 99, 100) (101)
	Proliferation of immune cells	(53)
	Atherosclerotic plaque instability and rupture	(86, 92–98)
	Myocardial infarction	(26–29, 111, 113, 153)
	Cardiac dysfunction	(134, 154)
	Anti-inflammatory function	(118–134)
	Pro-resolving function	(29)