# The Role of DAMPs and PAMPs in Inflammation-mediated Vulnerability of Atherosclerotic Plaques

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<td>cjpp-2016-0664.R2</td>
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<td>Review</td>
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<td>Date Submitted by the Author:</td>
<td>29-Dec-2016</td>
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<td>Complete List of Authors:</td>
<td>Rai, Vikrant; Creighton University School of Medicine, Department of Clinical &amp; Translational Science Agrawal, Devendra; Creighton University School of Medicine, Department of Clinical &amp; Translational Science</td>
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<td>Is the invited manuscript for consideration in a Special Issue?:</td>
<td>IACS Sherbrooke 2016 special issue Part 2</td>
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<td>Keyword:</td>
<td>Inflammation, Atherosclerotic Plaque, DAMPs, PAMPs, Plaque Vulnerability</td>
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The Role of DAMPs and PAMPs in Inflammation-mediated Vulnerability of Atherosclerotic Plaques

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Running title: DAMPs, PAMPs and plaque vulnerability
Abstract

Atherosclerosis is a chronic inflammatory disease resulting in the formation of the atherosclerotic plaque. Plaque formation starts with the inflammation in fatty streak and progress through atheroma, atheromatous plaque, and fibroatheroma leading to development of stable plaque. Hypercholesterolemia, dyslipidemia, hyperglycemia are the risk factors for atherosclerosis. Inflammation, infection with viruses and bacteria, and dysregulation in the endothelial and vascular smooth muscle cells leads to advanced plaque formation. Death of the cells in the intima due to inflammation results in secretion of damage-associated molecular patterns (DAMPs) such as high mobility group box 1 (HMGB1), receptor for advanced glycation end products (RAGE), alarmins (S100A8, S100A9, S100A12, and oxidized low-density lipoproteins), and infection with pathogens leads to secretion of pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides, lipoteichoic acids, and peptidoglycans. DAMPs and PAMPs further activate the inflammatory surface receptors such as TREM-1 and TLRs and downstream signaling kinases and transcription factors leading to increased secretion of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and interferon (IFN)-γ and matrix metalloproteinases (MMPs). These mediators and cytokines along with MMPs render the plaque vulnerable for rupture leading to ischemic events. In this review, we have discussed the role of DAMPs and PAMPs in association with inflammation-mediated plaque vulnerability.

Keywords: Inflammation; Atherosclerotic arterial plaque; DAMPs; PAMPs; Oxidized-LDL; Plaque vulnerability
1. Introduction

Atherosclerosis, a chronic disease characterized by the deposition of excessive lipids in the arterial intima is associated with inflammation, hyperglycemia, hyperlipidemia, and dysregulation of the angiotensin system (Xu et al. 2016). Aggregation and oxidation of the deposited lipids in the intima leads to the generation of modified lipid moieties (minimally-oxidized lipid, oxLDL) further causing the chronic stimulation of the innate and adaptive immune response. Induction of endothelial cells and vascular smooth muscle cells leads to expression of adhesion molecules, chemoattractants, and growth factors. Interaction of these factors with receptors on monocytes results in homing, migration, and differentiation of these monocytes into macrophages and dendritic cells, and secretion of pro-inflammatory cytokines involving the toll-like receptors (TLRs) (Bentzon et al. 2014) (Figure 1).

Atherosclerotic plaques consist of thick sclerotic collagen-rich fibrous cap and a lipid-rich atheromatous mass. Increases in plaque size, intra- and extracellular lipid accumulation, intra-plaque rupture, and inflammation renders the stable plaque unstable. Vulnerable plaques are described as the plaque prone to rupture and susceptible for thrombus formation and ischemic events (Butcovan et al. 2016). Infiltration of the inflammatory cells and secretion of the pro-inflammatory cytokines enhances the immune response and inflammation in the plaque. Increased level of inflammation in the plaque has been associated with plaque vulnerability (Ridker and Luscher 2014). Vulnerable plaques are characterized by the presence of a thin inflamed fibrous cap, a lipid core, necrotic core, increased neovascularization, inflammation, changes in vessel media and adventitia, intraplaque hemorrhage, and positive vascular remodeling (Otsuka et al. 2014; Virmani et al.)
Further, the serum inflammatory markers and the oxidized LDL, cholesterol, apolipoprotein B, homocysteine levels and the matrix metalloproteinases (MMPs) levels have been discussed as the serum markers of vulnerabilities (Benedek et al. 2016; Chan and Watts 2006; Koening et al. 2011; Minamisawa et al. 2016; Mittal et al. 2014; Sreckovic et al. 2016).

Increased inflammation and lipid accumulation render the plaque unstable. Exposure of the macrophages to apoptotic bodies and necrosis in the plaque resulting in the necrotic core formation leads to the release of damage-associated molecular patterns (DAMPs) such as high mobility group box 1 (HMGB1). Increased HMGB1 and pro-inflammatory cytokines secreted from pro-inflammatory cells feedbacks the cycle and further increases the pro-inflammatory cytokines and HMGB1 (Butcovan et al. 2016). Increased HMGB1 enhances the inflammation through TLRs, receptor for advanced glycation end products (RAGE), triggering receptors expressed on myeloid cells (TREM)-1, and increased pro-inflammatory cytokines production such as interleukin (IL)-6, IL-1β (beta), and tumor necrosis factor alpha (TNF-α) (Andersson and Tracey 2011; Pelham and Agrawal 2014) (Figure 2). Since increased inflammation renders the stable plaque unstable; these mediators of inflammation may play a potential role in vulnerability of atherosclerotic plaque. The role of TREM-1 and TLRs involving NF-κB and pro-inflammatory cytokines TNF-α in the pathogenesis of unstable plaque has been documented (Gargiulo et al. 2015; Rai et al. 2016; Rao et al. 2016a; 2016b).

Along with inflammation, the role of infection of the arterial intima and the presence of viruses and bacteria and their products has been discussed in association with the plaque development, progression and vulnerability (Lanter et al. 2014; Rosenfeld and Campbell 2011; Zimmer et al. 2015). Inflammation of the intima leads to damage of the endothelial
and vascular smooth muscle cells and production of DAMPs such as HMGB-1, RAGE, endogenous receptor ligand for advanced glycation end products (EN-RAGE), and alarmins (calgranulins). The deposition of the lipids and oxLDL causes activation of innate and adaptive immunity leading to increased production of inflammatory cytokines and homing of inflammatory cells. Infection of the arterial intima with viruses and bacteria results in the production of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS), peptidoglycans, and lipoteichoic acid (Chen et al. 2013; Kim et al. 2013; Thankam et al. 2016) (Figures 2 and 3). Here, we have discussed the role of DAMPs and PAMPs and their interaction with inflammatory mediators in the pathogenesis of inflammation-mediated plaque vulnerability.

2. Damage-associated molecular Patterns

2.1 High Mobility Group Box 1

HMGB1 is a highly conserved protein released by the injured or necrotic cells, and by the immune cells in response to inflammation and infection (Andersson and Tracey 2011). HMGB1 plays a role in sterile infection as well as in infectious inflammation. Exposure of the monocytes, macrophages, dendritic cells, endothelial cells, and platelets to pro-inflammatory cytokines such as TNF-α, IL-1β, and interferon-gamma (IFN-γ), microbe-associated molecular patterns (MAMPs), and PAMPs leads to the secretion of DAMPs such as HMGB-1. Further, the secretion of HMGB-1 is increased by a feed-forward loop by the secreted HMGB-1, and due to the damage and apoptosis of the cells in intima and exposure of the monocytes with apoptotic bodies (Andersson and Tracey 2011). Increased HMGB-1 induces the production of inflammatory cytokines (IFN-γ, TNF-α, IL-1β, IL-6) contributing a potential role in the progression of atherosclerosis and plaque vulnerability (Su et al. 2015).
(Figure 2). The development of advanced plaque in the rabbit model of atherosclerosis with the administration of HMGB-1 and TNF-α suggests the role of pro-inflammatory cytokines and HMGB-1 in increasing the inflammation and development of advanced plaque (Kim et al. 2016).

Metabolic syndrome, a low-grade chronic inflammatory state, affects 35% of US adults and consists of increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels. It is associated with increased risk for cardiovascular disease, and increased expression of the pathogen recognition receptors, toll-like receptors (TLRs). The increased levels of TLRs (TLR2 and 4) may be due to the increased circulating levels of PAMP-binding proteins (soluble CD14 and lipopolysaccharide binding protein), and the DAMPs (HMGB-1) (Jialal et al. 2014; Jialal et al. 2015). The increased levels of DAMPs and PAMPs contribute to TLRs mediated pro-inflammatory response, a major factor associated with the pathogenesis of atherosclerosis. Hypercholesterolemia promotes pro-inflammatory cytokine production and plaque destabilization by inducing IL-1β, IL-8, TNF-α, S100A8, S100A12 (ENRAGE), and MMP1 through Syk/PI3K and NF-κB signaling (Corr et al. 2016; Gargiulo et al. 2015) (Figures 2 and 3). Hyperglycemia regulates the release of HMGB-1 from leukocytes which facilitates the thrombus formation (Yamashita et al. 2012). Activated platelets have a potential role in increasing inflammation and in the development; progression and transition of stable to unstable plaque and binding of HMGB-1 with platelets involving RAGE further enhance the inflammation and progression of plaque (Ahrens et al. 2015).

Increased HMGB-1 is associated with induction of matrix metalloproteinases (MMPs) and matrix degradation resulting in collagen loss and blockade of HMGB-1 results in
decreased inflammation and MMP activity (Kohno et al. 2012). Increased MMP activity is
associated with plaque vulnerability and blocking MMPs may be a novel strategy and a
promising target for stabilizing vulnerable plaques in patients with carotid stenosis (Rao, et
al. 2014). Further, the TREM-1 (Rao et al. 2016a) and TLR4 (Gargiulo et al. 2015) mediated
regulation of MMPs via TNF-α in carotid plaque vulnerability, and the role of TREM-1,
HMGB-1 and RAGE axis in inflammation has been discussed (Thankam et al. 2016)
suggesting the association of these mediators in inflammation mediated plaque
vulnerability.

2.2 Receptor for Advanced Glycation End-products

RAGE is a pattern recognition receptor and mediates the innate immune response.
RAGE is expressed on immune cells, T and B-lymphocytes, and dendritic cells, and is
involved in immediate inflammatory responses. The ligand for the RAGE is advanced
glycation end products (Kierdorf and Fritz 2013). The non-enzymatic reaction between
reducing sugars with free amino groups on proteins, lipids, or nucleic acids results in the
formation of a heterogeneous group of complex structures called as advanced glycation end
products (AGEs). Further, glucose and (or) fructose metabolism intermediate derived
glyceraldehydes-derived AGEs are called as toxic AGEs (TAGE) and play a role in
development and progression of plaque (Takeuchi 2016). AGE formation and accumulation,
a normal process of aging is accelerated by hyperglycemia in diabetes making it a major risk
factor for atherosclerosis (Xu et al. 2016). Increased concentration of AGEs, oxidative stress,
and inflammation facilitates the lipid deposition leading to development and progression of
atherosclerosis (Figure 2). AGEs play a potential role in the pathogenesis of atherosclerosis
via engagement with RAGE present on the various cells found in the atherosclerotic lesion.
The binding of the AGEs (commonly carboxymethyl lysine and methylglyoxal-derived hydroimidazolone-1) with RAGE results in increased monocyte activation and macrophage migration, thereby increasing the inflammation in the arterial wall (Heier et al. 2015) (Figures 2 and 3). Further, AGEs-RAGE engagement also increases the lipid accumulation in macrophages by regulating cholesterol uptake, esterification, and efflux, thereby increasing the foam cells and atherogenesis (Xu et al. 2016).

RAGE also increases the lipid deposition and foam cell formation in smooth muscle cells by smooth muscle cell priming and increasing the uptake of minimally oxidized low-density lipoprotein (Chellan et al. 2016). Further, as discussed earlier, increased deposition of lipids, oxidative stress, and inflammation are the characteristics of unstable plaque. RAGE being a mediator of these factors plays a potential role in the destabilization of plaque. Vascular smooth muscle cell and endothelial cell dysfunctions are associated with atherogenesis and unstable plaque, and RAGE plays a role in AGES-induced vascular smooth muscle cell (VSMC) dysfunction (Nam et al. 2016). This suggests that RAGE is a potential mediator of plaque vulnerability.

Endothelial dysfunction is associated with atheroma formation. Endothelial progenitor cells are essential for re-endothelialization and neovascularization, and the paucity and dysfunction of these cells have been associated with the pathogenesis of atherosclerosis (Chen et al. 2016; Yamashita et al. 2012). Hyperactivity of JNK signaling pathway mediated by AGEs-RAGE engagement results in increased reactive oxygen species and oxidative stress leading to cellular apoptosis and inhibition of cell proliferation (Chen et al. 2016). The transition of stable to unstable plaque is also mediated by increased inflammation by homing of inflammatory cells and pro-inflammatory cytokines.
Inflammatory mediators such as HMGB1, TLRs, TREM-1, S100A8, S100A9, and S100A12 play a potential role in increasing inflammation through inflammatory cytokines in the plaque, thereby increasing the vulnerability (Oesterle and Bowman 2015) (Figures 2 and 3).

RAGE is the receptor for not only AGEs, HMGB-1 (Bangert et al. 2016), TLRs (Medeiros et al. 2014), and TREM-1 (Zysset et al. 2016) but also for the calcium binding S100/calgranulin proteins (S100A8, S100A9, and S100A12) (Oesterle and Bowman 2015), and plays a potential role in inflammation-induced atherosclerosis and destabilization of plaque (Figure 3). This suggests that RAGE axis is a mediator for inflammation-induced development and progression of plaque as well as instability. Further, it has also been documented that soluble RAGE (sRAGE) works as the decoy receptor and decrease the RAGE signaling, thereby decreasing the inflammation. Increasing levels of sRAGE have been associated with the protective effect on inflammation but not on arterial wall thickness and stiffness, suggesting a potential therapeutic target and the role of RAGE in plaque vulnerability (Heier et al. 2015).

2.3 EN-RAGE (S100A12)

EN-RAGE is a member of the S100 protein (calcium-binding proteins) family (Foell et al. 2003). EN-RAGE is an endogenously produced inflammatory ligand of the RAGE and Toll-like receptor 4 and endogenous receptor ligand for AGEs. The binding of RAGE with EN-RAGE activates inflammatory cascades involving NF-κB and inflammatory cytokines mediating inflammation and atherosclerosis (Ligthart et al. 2014). The significant association between the increased levels of S100A12 and atherosclerotic cardiovascular disease (Shiotsu et al. 2011) and with carotid atherosclerosis (Abbas et al. 2012) has been reported.
Abbas et al. (2012) reported the association of the highest increase in S100A12 plasma levels with early symptoms (2 months), whereas only the modest increase in S100A8/S100A9 plasma levels at 2 to 6 months but not later. The increased mRNA levels of S100A8, S100A9, and S100A12 were also associated with the carotid plaque development. Further, the TLR2 and TLR4 activation increases the mRNA expression of S100A8, S100A9, and S10012 and increases the release of interleukin-1β and interferon-γ from thrombin-activated platelets leading to significantly enhanced expression of S100A12 (Figure 3). This suggests the pathogenic role of S100A12 in association with other inflammatory mediators in the early phase of development.

However, the pleiotropic role of S10012A has also been reported. Higher levels of S100A12 were found to be associated with plaque rupture and percutaneous coronary intervention, but no stimulation of pro-inflammatory cytokines and inhibition of MMP-2, MMP-9, and MMP-3 via Zn$^{2+}$ sequestration suggest the protective role of S100A12 in advanced atherosclerotic plaque (Goyette et al. 2009). Contrarily, reduced amount of infiltrating inflammatory cells, diminished intimal and medial vascular calcification, smaller necrotic cores, normalization of RAGE expression and delayed type hypersensitivity with a S100 protein binding immuno-modulatory compound (ABR-215757) by attenuating the S100A12 expression suggest its protective role on plaque instability, but atherogenic role of S100A12 (Yan et al. 2013). Furthermore, the strong association between neutrophil-derived human S100A12 with the increased risk of coronary heart disease suggests the potential role of S100A12 in atherosclerosis and plaque vulnerability (Ligthart et al. 2014; Liu et al. 2014).

2.4 Alarmins
2.4.1 S100A8 and S100A9 (calgranulins)

Alarmins S100A8 and S100A9 are the members of calcium-binding protein family calgranulins. S100A8 and S100A9 are markers of inflammation, and play a potential role in the pathogenesis of various inflammatory diseases (Vogl et al. 2014). In inflammatory states, S100A8 and S100A9 are expressed primarily by myeloid cells such as dendritic cells and by non-myeloid cells such as endothelial cells and vascular smooth muscle cells (VSMCs), and modulate the inflammatory processes through TLR4 and RAGE (Figure 3). LPS increases the expression of S100A8 and S100A9 in VSMCs. Increased levels of S100A8 and S100A9 have been associated with increased cardiovascular events and atherosclerosis (Averill et al. 2012). The accumulation of AGEs is associated with the increase in S100A8, S100A9, and IL-1β expression thereby increasing the inflammation (Nakajima et al. 2015). Further, the increased expression of S100A8 and S100A9 on monocytes, macrophages, and the human arterial wall has been associated with atherogenesis and rupture prone-plaque (Ionita et al. 2009; McCormick et al. 2005). Increased levels of S100A8 and S100A9 in the area of atherosclerotic plaque and thrombus are believed to be derived from the activated platelets (Lood et al. 2016).

Unstable plaque is characterized by the thin fibrous cap. Thinning of the fibrous cap is mediated by the degradation of the collagen content of the extracellular matrix by the matrix metalloproteinases (MMPs) resulting in plaque rupture and ischemic events (Butoi et al. 2016; Newby 2015; Rao et al. 2015). Further, it has also been documented that S100A8 and S100A9 are the inducers of MMPs (Moz et al. 2016; Schelbergen et al. 2012; van Lent et al. 2012) (Figure 3). Hence, increased alarmins with age and inflammation in the arterial plaque may induce MMPs and may mediate the collagen loss and plaque rupture. These
results suggest that S100A8 and S100A9 increases inflammation and induces MMPs mediating thinning of the fibrous cap and plaque rupture. Higher levels of these alarmins have been correlated with plaque rupture (Ionita et al. 2009; Schiopu and Cotoi 2013; Xia et al. 2016). However, studies have also reported that S100A8 and S100A9 inhibit MMPs (Isaksen and Fagerhol 2001) and may suppress innate immune response in dendritic cells (Averill et al. 2011). Therefore, further studies are needed to correlate the level of alarmins and MMPs activation in stable plaque rendering them unstable. Furthermore, higher levels of alarmins have been associated with plaque vulnerability, and studies are needed to determine the predictive power of alarmins in at-risk patients (Averill et al. 2011; Ionita et al. 2009; Isaksen and Fagerhol 2001; Schiopu and Cotoi 2013; Xia et al. 2016).

2.4.2 Oxidized low-density lipoprotein (oxLDL)

Oxidized LDL is a marker of cardiovascular disease and correlates with its risk and severity (Fraley and Tsimikas 2006). The oxLDL increases the accumulation of cholesterol within the foam cells of atherosclerotic lesions (Itabe et al. 2011). Oxidized-LDL enhances the endothelial dysfunction and foam cell formation and increases the atherogenesis involving the monocytes, macrophages, and mast cells through increased secretion of TNF-α and histamine (Chen and Khismatullin 2015; Cipolletta et al. 2005; Singh et al. 2002; Zhu et al. 2005). Mast cell granules also increase the development of foam cell by its binding with LDL and increasing the uptake of LDL by macrophage. Reduced progression of atherosclerosis, lipid deposition, and recruitment of T-lymphocyte and macrophage into the plaque in LDLr and mast cell-deficient (LDLr−/− KitW-sh/W-sh) mouse (Sun et al. 2007) suggest the role of mast cell-LDL interaction in the progression of atherosclerosis. These studies also suggest that mast cell deficiency markedly reduces the progression of atherosclerosis. The
co-activation of macrophage and mast cell by higher circulating levels of oxLDL enhances the risk of atherosclerosis (Chen and Khismatullin 2015). Since oxLDL can be formed in the liver as well as in the atherosclerotic lesion, the circulating oxLDL and the oxLDL produced in the plaque affect the plaque vulnerability differently (Itabe et al. 2011). Niccoli et al. (2007) reported that neither oxLDL (recognized by monoclonal antibody mAb-4E6) nor malondialdehyde-modified LDL (recognized by monoclonal antibody mAb-1H11) in the plaque plays a role in plaque instability; however circulating oxLDL may play a role in plaque vulnerability. Similar to oxLDL, oxidized-cholesterol was also found to be more hypercholesterolemic and atherogenic than cholesterol in hamster (Ng et al. 2008).

Hypercholesterolemia, dyslipidemia, and inflammation are risk factors for atherosclerosis and plaque vulnerability (Gupta et al. 2016). Dyslipidemia is also associated with the increased expression of inflammatory mediator TREM-1 in advanced plaque (Zysset et al. 2016). Further, increased TREM-1 expression is associated with unstable plaque and plaque vulnerability. This suggests that dyslipidemia enhances the inflammation in plaque through increased surface expression of TREM-1 leading to increased monocytes homing, production of pro-atherogenic or pro-inflammatory cytokines, and foam cell formation, and thus enhanced the plaque vulnerability (Rai et al. 2016; Rao et al. 2016a; Zysset et al. 2016) (Figures 2 and 3). Increased expression of TREM-1 on matured dendritic cells has been correlated with plaque instability (Rai et al. 2016), and DCs and activated T cells have been co-localized in atherosclerotic plaque. Further, suppression of the atherogenic potential of the oxLDL induced DCs and limitation of the pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) production with statins suggest the role of oxLDL in atherogenesis and plaque vulnerability (Frostegard et al. 2016). Similarly, decreased expression of TNF-α and oxLDL-
induced inflammatory cytokines with soluble-TREM-1 suggests the potential role of TREM-1 and oxLDL in coronary artery disease and atherosclerosis (Dai et al. 2016).

TREM-1 amplifies the TLR4 signaling and enhances the inflammation and immune reaction (Pelham and Agrawal 2014). TLRs participate in lipid deposition and enhanced inflammation in atherogenesis. The expression of TREM-1 and TLR4 in macrophage can be increased by oxLDL leading to increased pro-inflammatory cytokine production. Inhibition of TREM-1 with short-hairpin RNA and synthetic polypeptide LP-17 leads to decreased secretion of pro-inflammatory cytokines, suggesting the potential role of oxLDL induced TREM-1 and TLR4 in atherosclerotic plaque development, progression, and vulnerability (Li et al. 2016). The role of oxLDL mediated TLR4 activation in macrophages, and association of increased expression of TLR4 with increased inflammation suggests the correlation of TLR4, oxLDL, inflammation, and atherosclerosis (Xu et al. 2001). Since, increased expression of inflammatory mediators (TREM-1, TLRs, and inflammatory cytokines), dyslipidemia, and inflammation is also associated with unstable plaque; activation of TLR4 with oxLDL may be correlated with plaque vulnerability (Miller 2005; Rai et al. 2016; Rao et al. 2016a; 2016b).

3. Pathogen Associated Molecular Patterns

Along with inflammation, infection of the intima may also play a role in the development, progression and plaque destabilization. The presence of bacteria and viruses in the atherosclerotic area has been documented (Lanter et al. 2014; Rosenfeld and Campbell 2011; Zimmer et al. 2015). Direct infection of vascular cells, production of endotoxins, modulating the immune response, secretion of pro-inflammatory cytokines or acute phase proteins, destruction of the intima or activation of the mediators of inflammation by these viruses and bacteria are the possible mechanism contributing to
atherogenesis (Lanter et al. 2014; Rosenfeld and Campbell 2011; Zimmer et al. 2015). Further, the presence of bacteria as biofilm proximal to the internal elastic lamina and in association with fibrous tissue has been found, and rupture or dispersion of this biofilm is associated with the increased propensity of plaque rupture (Lanter et al. 2014). Chronic inflammation plays a potential role in atherogenesis and increased inflammation in the plaque induces the plaque instability. HMGB-1 secretion from the necrotic core and by positive feedback enhances this process through secretion of the pro-inflammatory cytokines. HMGB-1 expression and release can also be increased by bacterial endotoxins lipopolysaccharide, a known pathogen-associated molecular patterns (PAMPs) (Chen et al. 2013). As discussed above, the interaction of HMGB-1 with TREM-1, TLRs, and RAGE result in increased secretion of pro-inflammatory cytokines, inflammation, and plaque vulnerability. Further LPS exposure increases expression of HMGB-1 and inflammation rendering the plaque more vulnerable (Jaw et al. 2016) (Figure 3). Increased TREM-1 expression on macrophages with LPS, TLR4 mediated TREM-1 expression, LPS mediated increased TLRs expression, and involvement of HMGB1-RAGE axis and LPS binding protein in increasing inflammation suggests the potential role of LPS in enhancing the expression of inflammatory mediators (Arts et al. 2011; Huebener et al. 2015; Murakami et al. 2007; Park and Lee 2013; Pelham and Agrawal 2014; Wu et al. 2012) (Figure 3). Since these mediators are involved in the pathogenesis of plaque instability, LPS may play a potential in plaque destabilization.

An aberrant vascular smooth muscle proliferation result in plaque formation, however, VSMCs regeneration is essential for the repair of the plaque area (Bennett et al. 2016). Reduced regenerating power of VSMCs renders the plaque unstable and is a
characteristic feature of unstable plaque. VSMCs apoptosis, cell senescence, and VSMC-derived macrophage-like cells may promote inflammation, progression, and destabilization of the plaque (Bennett et al. 2016). VSMCs associated plaque progression and instability is mediated by the DNA damage products called double-stranded breaks, which promotes cell senescence, apoptosis, and inflammation (Bennett et al. 2016; Gray et al. 2015). Activation of Na\(^+\)/H\(^+\) exchanger-1 by LPS via Ca\(^{2+}\)/calpain results in apoptosis of VSMCs apoptosis and atherosclerosis and inhibition of Na\(^+\)/H\(^+\) exchanger-1 leads to attenuation of LPS-accelerated atherosclerosis and promotes plaque stability, suggesting the potential role of LPS in plaque destabilization (Li et al. 2014).

Rupture of the plaque is mediated by the destruction of the extracellular membrane by MMPs (Newby 2008). Newby (2015) reviewed the role of MMPs in atherosclerosis and concluded the stabilizing effect of MMPs on intimal thickening leading to plaque stabilization, as well as the destabilizing effect on the plaque by extracellular matrix and collagen degradation (Newby 2008; Newby 2015). Further, LPS induced stimulation of monocytes and production of MMP-1 and MMP-9 has been documented in association with the ischemic events and plaque destabilization (Speidl et al. 2004). Furthermore, both TLR4 and TREM-1 are mediators of inflammation and the role of LPS in enhancing the MMP-9 expression through TLR/NF-κB stimulation, and TREM-1 expression through TLR4 dependent pathway, in the context of atherosclerosis has been reported (Li et al. 2012; Zheng et al. 2010). The correlation of increased expression of MMP-1, and MMP-9, TREM-1, and decreased expression of collagen I and collagen III through involvement of TNF-α, epidermal growth factor, Ets-1, NF-kB, p38-MAPK, JNK-MAPK, and PI3K in relation to plaque destabilization has also been documented (Rao et al. 2014; Rao et al. 2015; Rao et al. 2016a)
These results suggest the potential role of LPS in carotid plaque destabilization via stimulation of these mediators of inflammation.

The role of PAPMs such as lipoteichoic acid and peptidoglycans has also been discussed in the literature in relation to the atherogenesis and the plaque vulnerability (Kim et al. 2013; Laman et al. 2002; Schoneveld et al. 2005). Lipoteichoic acid suppresses the LPS induced inflammation in the plaque by attenuating the expression of MMP-9, COX-2, Bax, HSP27, TLR4 and CCR7 through downregulation of NF-κB along with inhibiting the monocyte/macrophage infiltration (Kim et al. 2013). Peptidoglycan present in atherosclerotic plaque is a functional LPS analog which induces pro-inflammatory cytokine production and MMPs via binding to CD14 on macrophages. Significantly increased numbers of the peptidoglycan positive cells have been correlated with the vulnerable plaque (Laman et al. 2002). Peptidoglycan stimulates TLR2 and enhances the inflammation and plaque vulnerability (Schoneveld et al. 2005). Further, amplification of mRNA and protein expression of IL-6, TLR2 and TLR4 increased the secretion of IL-6, IL-8, and nuclear translocation of NF-κB in the human umbilical vein endothelial cells incubated with LPS, lipoteichoic acid, or peptidoglycan in the presence of histamine (Jehle et al. 2000; Talreja et al. 2004). These findings suggest a synergy between PAMPs and endogenously secreted histamine in activating the endothelial cells resulting in enhanced production of inflammatory cytokines (Jehle et al. 2000; Talreja et al. 2004) (Figures 2 and 3).

Endothelial dysfunction plays a crucial role in plaque development and vulnerability (Raveendran et al. 2011; Tan et al. 2007). In response to increased inflammation, endothelial cells may also enhance secretion of the inflammatory cytokines and activation of mediators of inflammation, thereby contributing to plaque progression and rupture.
(Raveendran et al. 2011; Tan et al. 2007). The synergy between endogenously released histamine and LPS in increasing the inflammation via increased mRNA expression of H1 receptors resulting in increased production of prostaglandin I2, prostaglandin E2 and IL-6 by endothelial cells has been reported. This effect was partly attributed to histamine-induced expression of TLR4 (Raveendran et al. 2011) (Figures 2 and 3). Production of prostaglandin I$_2$ and prostaglandin E$_2$ can also be increased by stimulating the cyclooxygenase-2 through LPS and histamine (Tan et al. 2007). These studies suggest that histamine release in endothelial cells increases inflammation and may contribute to plaque vulnerability. The role of flagellin-induced activation of NADPH oxidase-4 involving TLR5 and production of hydrogen peroxide has been discussed. This lead to increased secretion of pro-inflammatory cytokines and adhesion molecule leading to increased inflammation, reduction in the adhesion and transendothelial migration of monocytes, and atherosclerosis (Kim et al. 2016). Since monocytes and macrophages play a potential role in inducing inflammation and plaque destabilization, flagellin-induced inflammation may play a potential role in plaque vulnerability (Kim et al. 2016).

4. Conclusion

Atherosclerotic plaque development, progression, and vulnerability are affected by inflammation and infection by viruses and bacteria and their products. Inflammation and infection in the fatty streak result in homing of inflammatory cells, increased apoptosis and necrosis, and increased lipid deposition leading to secretion of DAMPs and PAMPs, and inflammatory cytokines involving surface receptors and downstream signaling molecules (Lanter et al. 2014; Rosenfeld and Campbell 2011; Thankam et al. 2016; Zimmer et al. 2015) Increased secretion of the inflammatory cytokines and matrix metalloproteinases mediate
the transition of stable plaque to unstable, thus increasing the plaque vulnerability (Rao et al. 2014; Rao et al. 2016a; Rao et al. 2016b). As discussed, these mediators of inflammation serve as the markers for the assessment of the extent of plaque vulnerability as well as potential therapeutic targets for the vulnerable plaque. Decreased secretion of inflammatory cytokines and morphological features of unstable plaque with blocking HMGB1, the use of sRAGE and sTREM1, and TREM-1 blocking with LP17 and short hairpin RNA suggest the potential therapeutic options. The pleiotropic effect of EN-RAGE and oxLDL (plaque vs. circulatory), differential expression of S100A12 and S100A8/A9 with time has been discussed but needs further research. Further understanding the effect of DAMPs and PAMPs on VSMCs and endothelial cells, analyzing the protective effect of lipoteichoic acid by attenuating LPS action, and elaborating the effects of plaque and circulatory oxLDL would be therapeutically beneficial.

Acknowledgement

This work was supported by research grants R01 HL112597, R01 HL116042, and R01 HL120659 to DK Agrawal from the National Heart, Lung and Blood Institute, National Institutes of Health, USA. The content of this review article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Financial and competing interests’ disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with financial interest or financial conflict with the subject matter or
materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

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**Figure legend**

**Figure 1:** Pathogenesis of the development of atherosclerotic plaque. Fatty streaks are the initial lesions in the process. The inflammation and oxidative stress in the fatty streaks and lipid deposits lead to the formation of atheroma with increased VSMCs proliferation and homing of inflammatory cells. Innate immune response results in the production of inflammatory cytokines and increased inflammation in atheroma. Apoptosis and necrosis of the cells result in secretion of damage associated molecular patterns (DAMPs), which further activate the inflammatory mediators resulting in the formation of necrotic core. Thus, atheroma progresses through atheromatous plaque to fibro-atheromatous plaque with increased inflammation and inflammatory cytokines. This leads to thinning of fibrous cap, endothelial dysfunction, angiogenesis, VSMCs proliferation and collagen damage resulting in the destabilization of the plaque to become unstable.

oxLDL - Minimally oxidized-low density lipoproteins; RBCs - red blood cells, VSMCs - vascular smooth muscle cells.

**Figure 2:** Progression of atherosclerotic plaque. Increased deposition of the oxLDL and AGEs induces the innate immune response resulting in increased homing of inflammatory cells and secretion of inflammatory cytokines, such as IL-6, TNF-α, IFN-γ and IL-1β, leading to formation of apoptotic bodies and necrotic core and enhanced inflammation in plaque area. LPS, bacterial endotoxins and endogenously released substance such as histamine also enhance inflammatory cytokines and other mediators of inflammation. HMGB-1 released from these dying cells activates the inflammatory cascade through TREM-1, TRLs and RAGE leading to enhanced inflammation, inflammatory cytokine secretion, activation of MMPs, collagen loss, angiogenesis and hemorrhage. VSMCs proliferation, calcification, necrotic core
and thinning of the fibrous cap render the plaque vulnerable to rupture. AGEs - Advanced glycation end products, HMGB-1 - high mobility group box-1, IL-6 – interleukin-6, IL-1β – interleukin-1 beta, IFN-γ- interferon-gamma, LPS - lipopolysaccharides, ox-LDL - minimally oxidized-low density lipoproteins, MMPs - matrix metalloproteinases, RAGE - receptor for advanced glycation end products, TREM-1 - triggering receptor expressed on myeloid cells, TLRs - toll-like receptors, TNF-α - tumor necrosis factor-alpha, and VSMCs - vascular smooth muscle cells.

**Figure 3: Role of DAMPs and PAMPs in inflammation-mediated plaque vulnerability.**

HMGB1 secreted from necrotic cells and from the interaction of apoptotic bodies with inflammatory cells interacts with RAGE, TREM-1 and TLRs to activate them. Activation of these inflammatory receptors initiates a downstream signaling pathway involving NF-κB resulting in increased secretion of inflammatory cytokines such as IL-6, TNF-α, IFN-γ and IL-1β. This leads to further increased inflammation, activation of MMPs, collagen loss and plaque rupture. Accumulation and oxidation of AGEs and their interaction with RAGE and EN-RAGE, and accumulation of alarmins S100A8 and S100A9 and interaction with TLRs enhance inflammatory cytokine production and plaque vulnerability. LPS-induced HMGB-1, TREM-1, TLRs, and alarmins and peptidoglycans-induced activation of TLRs also increase inflammatory cytokines and plaque vulnerability. AGEs - Advanced glycation end products, EN-RAGE - endogenous receptor for advanced glycation end products, ECM - extra cellular matrix, HMGB-1 - high mobility group box1, IFN-γ - interferon gamma, IL-6 - interleukin-6, IL-1β - interleukin-1beta, LPS - lipopolysaccharides, MMPs - matrix metalloproteinases, oxLDL - minimally oxidized lo density lipoprotein, NF-kB - nuclear-factor kappa beta, RAGE -
receptor for advanced glycation end products, TLRs - toll-like receptors, TREM1 - triggering receptor expressed on myeloid cell1, TNF-α - tumor necrosis factor alpha.
oxLDL accumulation, release of chemoattractant and growth factors

↑ Homing of inflammatory cell and secretion of inflammatory cytokines

Increased inflammation, necrotic core formation, angiogenesis, and hemorrhage

Endothelial dysfunction, VSMCs proliferation, impaired repair and plaque rupture

Fatty Streak → Atheroma → Atheromatous plaque → Fibro-atheromatous plaque → Ruptured plaque

Apopotic cell

Adhesion molecules, chemoattractants, and growth factors

Angiogenesis

Endothelial cell

Monocyte

Foam cells

Macrophages

Hemorrhage

Necrotic core

Lipids

oxLDL

Platelets

RBCs

Thrombus

VSMCs
LPS

HMGB1

AGEs

S100A8 and S100A9

Cytoplasmic Kinases

↑ NF-κB

↑ MMPs

↑ Inflammatory cytokines (TNF-α, IL-1β, IL6, IL-8, IFN-γ)

Collagen and ECM degradation

↑ Plaque vulnerability

Lipids
Apoptotic bodies
oxLDL
Vascular smooth muscle cells
Macrophage
Foam cells
Monocytes
Apopotic cells
Hemorrhage
Necrotic core
Peptidoglycans
Bacteria
MMPs
AGEs