

NIH Public Access

Author Manuscript

Drug Discov Today Ther Strateg. Author manuscript; available in PMC 2009 May 7

Published in final edited form as:

Drug Discov Today Ther Strateg. 2008; 5(1): 15-23. doi:10.1016/j.ddstr.2008.05.007.

Targeting Innate Immunity for CV Benefit

Kathryn J. Moore and Mason W. Freeman

Lipid Metabolism Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

Abstract

The initiation and progression of vascular inflammation are driven by the retention of cholesterol in the artery wall, where its modification by oxidation and/or enzymes triggers the innate immune host response. Although previously considered a broad, primitive defense mechanism against invading pathogens, it has become clear that pattern recognition receptors of the innate immune system can cooperate to precisely regulate signaling pathways essential for the proper initiation of both innate and acquired immunity. Recent evidence suggests that these pattern recognition receptors may orchestrate the host response to modified endogenous ligands involved in sterile chronic inflammatory syndromes, including atherosclerosis. In this review we will summarize the current understanding of innate immune receptors and the putative ligands that regulate the numerous responses that promote this disease, including monocyte recruitment, macrophage cholesterol uptake, and pro-inflammatory signaling cascades. Specific emphasis will be placed on the potential of these innate immune targets for therapeutic interventions to retard the progression of atherosclerosis or to induce its regression.

Keywords

pattern recognition receptor; atherosclerosis; innate immunity; macrophage; scavenger receptor; chemokine; Toll-like receptor

Atherosclerosis is an inflammatory disease that develops in the context of hypercholesterolemia (reviewed in [1-3]). The current paradigm suggests that atherogenic lipoprotein particles, such as LDL and other apo B-containing lipoproteins, cross the endothelial cell barrier and accumulate in the subendothelial intima where they are subject to modification by oxidation and/or enzymatic action. Although the precise nature of the lipoprotein modifications remains a matter of debate, it is believed that these modified lipoproteins provoke an innate immune host response that kindles inflammation in the artery wall and drives plaque formation. This evolutionarily ancient host defense system is the body's first line of defense against invading pathogens and modified host ligands. Unlike adaptive immunity that relies upon clonal expansion of cells that emerge days after antigenic challenge, the innate immune response is immediate. Four main components contribute to the innate immunity; 1) soluble mediators, such as complement and natural antibodies, 2) epithelial barriers, 3) cellular defense including phagocytosis, and 4) germ-line encoded pattern recognition receptors (PRRs) that identify conserved molecular structures normally absent in the healthy host. This allows for a rapid response, typically characterized by ligand binding,

correspondence author: Freeman, M.W. (E-mail: freeman@molbio.mgh.harvard.edu).

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phagocytosis, and activation of conserved signaling cascades that initiate expression of inflammatory mediators such as cytokines and reactive oxygen species. In this review, we will concentrate on components three and four of the innate immune response, as it is these that appear to be directly involved in atherogenesis and may have, therefore, have the potential for therapeutic manipulation in the treatment of coronary artery disease.

Monocyte/macrophages

The macrophage, a major cellular effector of the innate immune response, is the predominant cell type in the early atherosclerotic lesion. This gives the macrophage a unique and primal role in the development and progression of atherosclerosis. Macrophages recognize and internalize modified lipoproteins leading to cellular cholesterol accumulation. Although initially beneficial, this protective response appears to become overwhelmed, leading to massive cellular cholesterol accumulation and the trapping of lipid-laden macrophages in the intima. These cholesterol-laden macrophage "foam cells" define the early atherosclerotic lesion pathologically and form what is known as fatty streak lesions. The release of cytokines, chemokines, reactive oxygen species and proteolytic enzymes by these accumulated macrophage foam cells modulates the progression of atherosclerotic lesions, including the recruitment of additional cellular components (T cells and smooth muscle cells), the deposition/ degradation of extracellular matrices, necrotic core formation and plaque rupture [1]. Thus, the recruitment of macrophages to the artery wall is a key event of the innate immune response in early atherogenesis and recent studies have shed new light on that process.

Monocyte recruitment and innate immune targets

Central to the development of atherosclerotic lesions is the influx of monocytes into the arterial intima and the initiation of a chronic inflammatory milieu that drives plaque formation. While monocyte recruitment is often cited as an early event in atherosclerosis, and therefore not considered an ideal target for the development of therapeutics in humans with established disease, it is very likely that monocyte ingress and egress are dynamic processes that actively contribute to lesion progression throughout all stages of coronary atherosclerosis. A newly recognized component of this macrophage trafficking is the selective recruitment of a subset of monocytes into atheromata [4,5]. Monocytes can be phenotypically divided into distinct subsets based on the expression of cell surface protein markers: Ly-6C^{hi} (Gr-1⁺CCR2⁺CX3CR1^{lo}) or Ly-6C^{lo} (Gr-1⁻CCR2⁻CX3CR1^{hi}) in mice and correspondingly, CD14^{hi}CD16⁻ or CD14⁺CD16⁺ in humans. Recent studies in *Apoe^{-/-}* mice indicate that a dramatic increase in the Ly-6Chi monocytes accounts for hypercholesterolemia-associated monocytosis [4,5]. Through chemokine receptor CCR2 and CX3CR1-dependent mechanisms. the subset of CCR2⁺ monocytes preferentially infiltrate the artery to become lesional macrophages [5]. Ly-6C^{lo} monocytes infiltrate lesions to a lesser extent and show increased expression of the dendritic cell marker CD11c [5]. These macrophage subsets are likely to exhibit distinct gene expression profiles that influence both cholesterol metabolism and inflammatory responses. While these remain to be defined, the identification of specific chemokines and chemokine receptors that modulate their recruitment into atherosclerotic lesions may help to refine targets to block monocyte entry into the artery wall without necessarily blocking monocyte recruitment to all sites and all stimuli of innate immunity. Recombinant decoy chemokines have shown some promise in attenuating atherosclerosis in Apoe^{-/-} mice. A deletion mutant of MCP-1 that blocks signaling via CCR2 and a modified RANTES peptide that blocks CCR5 signaling reduced lesion size in $Apoe^{-/-}$ mice, as did adenoviral-gene transfer of a 35 kDa vaccinia virus protein that acts as a pan inhibitor of CC chemokines [6-8].

Further studies will be required to determine if targeting monocyte recruitment will prove beneficial in more advanced atherosclerotic lesions characteristic of human disease. However, once in the arterial intima, macrophage expression of a number of receptors that are components of the innate response dictates the inflammatory response that ensues. Two families of PRRs that appear to have uniquely important roles in both the initiation and maintenance of vascular inflammation are known: the multi-ligand scavenger receptors (SR) and the microbial sensing Toll-like receptors (TLR). These families of pattern recognition receptors are actively being investigated as potential therapeutic targets in the treatment of atherosclerosis.

Scavenger receptors

The conversion of subendothelial macrophages into cholesterol-laden foam cells is believed to constitute the foundation of the atherosclerotic lesion. Native LDL has traditionally not been considered capable of generating foam cells because cells downregulate their LDL receptor number with increasing cellular cholesterol content, precluding lipid loading via this pathway [9]. Modification of LDL is therefore required to drive lipid uptake by alternative receptor pathways. Over the past thirty years, multiple means have been identified to alter LDL structure that facilitate its conversion of macrophages into lipid-laden foam cells. The widely accepted paradigm for the in vivo modification responsible for the alternative receptor binding behavior of LDL has been the oxidative modification hypothesis, most cogently proposed by Steinberg and colleagues in 1989 [10]. This theory posits that heightened oxidative stress in the vascular wall and its associated production of reactive oxygen and nitrogen species give rise to oxidized forms of LDL that are recognized by a family of scavenger receptors that we now recognize to be members of the innate immunity PRR class [11]. This family includes eight subclasses of structurally unrelated receptors that share the defining feature of being able to bind and internalize modified forms of LDL (Fig 1; reviewed in [12]). As suggested by their name, these receptors also recognize and "scavenge" other modified-self and non-self ligands, including bacteria, apoptotic cells, anionic phospholipids and amyloid proteins. The phagocytic function of these evolutionarily ancient receptors is believed to have its roots in tissue remodeling and host defense, however new evidence suggests that scavenger receptors can also direct cellular signal transduction either alone, or by pairing with dedicated signaling receptors such as the Toll-like receptors [12], which are discussed in greater detail below.

Scavenger receptors perform numerous functions that can modulate disease progression, including (1) recognition and internalization of modified lipoproteins, (2) induction of macrophage apoptosis, (3) clearance of apoptotic cells and debris, and (4) activation of cellular signal transduction [12]. To date, the majority of studies have focused on the role of scavenger receptors in macrophage foam cell formation. While many members of this family can bind native and modified LDL, including MARCO, CD68 (macrosialin), LOX-1, SR-BI and SREC, genetic loss-of-function studies have revealed substantial roles for SR-A, CD36 and CXCL16/ SR-PSOX in the formation of foam cells in vitro [13-16]. Macrophages derived from mice with targeted deletions in these receptors demonstrate 15%, 60%, and 30% reductions in oxLDL uptake, respectively [13-16]. However, in vivo atherosclerosis studies of SR-A, CD36, and CXCL16/SR-PSOX deficient mice have not uniformly shown a clear benefit to blocking these pattern recognition receptors, indicating that their roles in atherosclerosis are more complex than originally envisioned. While other scavenger receptors are likely to also contribute, the specific contribution of SR-A, CD36, and CXCL16/SR-PSOX to atherosclerosis in mouse models are summarized below and their potential as therapeutic targets discussed.

Scavenger receptor A

SRA was the first member of the scavenger receptor family to be molecularly identified and is expressed in monocytes/macrophages, smooth muscle and endothelial cells [17]. The role of SR-A in atherogenesis is currently controversial, as conflicting results have emerged from different groups studying the effect of targeted deletion and overexpression of SRA in multiple mouse models of atherosclerosis. Initial studies of SR-A deletion in the $Apoe^{-/-}$ mouse model on a hybrid background of ICR/129 showed a 58% decrease in aortic sinus lesion area compared to $Apoe^{-/-}$ littermates [16], while a second study in this same model in mice more highly backcrossed to the atherosclerosis-susceptible C57BL/6 strain revealed no benefit [18]. Subsequent studies were equally confounding: Msr deletion in the LDL receptor null $(Ldlr^{-/-})$ atherosclerosis model resulted in a reduction of atherosclerotic lesion size of approximately 25% [19], while in the apoE3 Leiden mouse a 35-85% increase in lesion area was noted [20]. Similarly confounding results have been reported with regard to the impact of overexpression of SRA on atherosclerosis. Transgenic overexpression of Msr in the Ldlr^{-/-} and $Apoe^{-/-}$ models did not exacerbate atherosclerosis in one study [21,22], while a second study of Msr overexpression in $Ldlr^{-/-}$ mice reduced atherosclerosis in the aortic arch by 74% [23]. The explanation for so much apparently conflicting data in mouse models of atherosclerosis is not clear, although it seems likely that the absence of a uniform genetic background from insufficient back-breeding of the original knock-out strain can contribute. Thus, although the use of decoy scavenger receptors has been suggested as a potential tool for therapeutic intervention in atherosclerosis, and at least one study in $Ldlr^{-/-}$ mice suggests that a secreted form of SR-A may prove beneficial for this purpose [24], the impact of blocking SRA activity in human atherosclerosis would be difficult to predict at this time.

CD36

CD36 is a member of the B class of the scavenger receptor family and unlike SRA, this receptor is widely expressed on monocytes/macrophages, adipocytes, microvascular endothelium, platelets, and erythroid precursors (reviewed in [12]). CD36 binds a diverse group of ligands that can contribute to atherosclerotic processes including endogenous ligands such as thrombospondin-1, collagen and fatty acids, as well as modified endogenous ligands such as oxidized LDL and apoptotic cells [12]. In addition to lipoprotein uptake, this receptor can initiate cellular signaling cascades regulating endothelial and macrophage responses, including cell survival, activation, and migration [25-28]. Furthermore, two recent studies demonstrated that CD36 can cooperate with Toll-like receptors to mediate the innate host response to *Staphylococcus aureus*, specifically phagocytosis and cytokine production [29,30]. Whether a similar cooperation regulates CD36 signaling by other ligands, such as oxidized LDL, is not yet known.

The preponderance of studies of CD36 deficiency in mouse models of atherosclerosis support a pro-atherosclerotic role for this receptor, however, this effect may be region-specific. Three studies performed in $Apoe^{-/-}Cd36^{-/-}$ mice have revealed reductions of 30-80% in atherosclerotic lesion area in the descending aorta, while cross-sectional analysis of aortic sinus lesions in these mice has resulted in findings ranging from a modest benefit to 20-40% increases in lesion size [14,18,31]. The reason for this disparity is not known, and the mechanism of CD36's contribution to atherogenesis remains to be clarified. $Cd36^{-/-}$ bone marrow transplantation studies into $Apoe^{-/-}$ mice support a pro-atherosclerotic role for macrophage expression of CD36 [32], however whether this is due to its function in lipid uptake, apoptotic cell clearance or signal transduction is not known. Interestingly, despite a lack of reduction in aortic sinus lesion area in $Cd36^{-/-}Apoe^{-/-}$ mice, lesions in this site were found to have reduced macrophage content and calcium deposition compared to $Apoe^{-/-}$ control mice [18,31]. Further investigation of the impact of CD36 deficiency on atherosclerotic plaque biology by immunohistochemical or transcriptomic analyses will be required to clarify the roles of this receptor in atherogenesis.

Despite these outstanding questions, therapeutic targeting of CD36 has been shown to be beneficial in the $Apoe^{-/-}$ mouse model of atherosclerosis. Subcutaneous injections with a CD36 ligand derived from growth hormone releasing peptide, EP80317, reduced established atherosclerotic lesion area in the aorta by up to 50% [33]. Furthermore, treatment of $Apoe^{-/-}$ mice with EP80317 for 12 weeks decreased total plasma cholesterol by 30%, suggesting potential effects of this drug on cholesterol metabolism, perhaps in the intestine or liver [33]. Additionally, naturally occurring antibodies that recognize oxidized LDL and *Streptococcus pneumoniae* block macrophage cholesterol loading via CD36, and immunization with *S. pneumoniae* reduces atherosclerotic burden in $Apoe^{-/-}$ mice [34]. These studies suggest that CD36 may be a promising target for modulation of early and established coronary artery disease.

CXCL16/SR-PSOX

The scavenger receptor CXCL16, also known as SR-PSOX, is unique in that it combines scavenger receptor functions with the properties of an inflammatory chemokine (reviewed in [12]). Membrane bound CXCL16 is composed of a glycosylated mucin stalk fused to a chemokine domain that attracts cells expressing the receptor CXCR6 and also mediates the internalization of oxLDL, bacteria and apoptotic cells. The extracellular domain of CXCL16 also undergoes proteolytic cleavage generating a soluble chemokine that activates CXCR6⁺ T helper 1 (Th1) cells. Thus this scavenger receptor may both promote macrophage foam cell formation and recruit effector T cells into the arterial intima. However, despite findings of a marked reduction in oxLDL uptake by *Cxcl16^{-/-}* macrophages *in vitro*, analysis of CXCL16 deficiency in *Ldlr^{-/-}* mice revealed a 30-45% increase in lesion area in the aortic arch lesion area and a 57% increase in the aortic sinus [13]. This increase in atherosclerotic burden was accompanied by greater macrophage recruitment and expression of the cytokines TNF α and MCP-1, but no difference in the total number of T cells [13]. Taken together, these data indicate blockade of CXCL16 is not a useful therapeutic target for patients with cardiovascular disease.

In aggregate, genetic-loss-of function studies of SR-A, CD36 and CXCL16 in mouse models of atherosclerosis have revealed a greater complexity to scavenger receptor biology than originally envisioned. As might be expected, these in vivo studies have uncovered a redundancy in pathways leading to macrophage foam cell formation. However, it has also become appreciated that the receptors by which lipoprotein particles are internalized may not only influence the amount of lipid taken up by macrophages, but also the inflammatory state of the macrophage. With the identification of additional roles for scavenger receptors in the regulation of apoptotic cell clearance, signal transduction, host response to pathogens and chemoattraction, it is now clear that these receptors may contribute to both pro- and antiinflammatory forces in the artery wall. However, the data generated from receptor knock-out mouse models of atherosclerosis using Western diet supplementation should also be interpreted with some caution. Western diet feeding conveniently accelerates atherosclerosis development in the mouse, thereby reducing the length of studies. The circulating cholesterol levels produced in Apoe^{-/-} or Ldlr^{-/-} mice on these diets are, however, much greater than those in the typical patient with coronary atherosclerosis and thus there may be pathways involved in human atherosclerosis that are rendered less relevant in the mouse models of the disease. For example, in the presence of such marked hypercholesterolemia, it is likely that the local concentrations of lipoproteins in the aortic intima may lead to saturating conditions that overwhelm scavenger receptors, triggering backup receptor pathways or non-receptor mediated lipoprotein uptake pathways. This could make the receptor pathways seem less important than they are in human atherosclerosis or, conversely, could elicit the involvement of receptor pathways whose affinity

for ligands would be too low to be activated in human arteries. Moreover, recent data indicates that unmodified LDL, at concentrations that are substantially higher than those needed to saturate high affinity receptor mechanisms but still achievable in hypercholesterolemic humans, can produce foam cells in the absence of scavenger receptors [18,35,36]. In summary, the relevance of scavenger receptor mediated mechanisms for macrophage cholesterol loading and inflammatory responses under more physiologic cholesterol levels will require further investigation before their suitability as therapeutic targets can be confidently assessed.

Toll-like receptors

Over the past decade the essential role of the Toll-like receptors in innate immune sensing of pathogens has become apparent (reviewed in [37]). This family of mammalian pattern recognition receptors has 11 members that act as homo- or hetero-dimers to recognize invariant patterns expressed on pathogens, including bacterial cell wall components and pathogen derived nucleotides. TLRs fall into two broad classes - the cell surface TLRs; TLR1/2/4/5/6 and the endosomal TLRs TLR3/7/8/9 (Fig. 2). The cell surface TLRs recognize ligands commonly exposed on the bacterial cell wall such as LPS and flagellin. In contrast, the endosomal TLRs recognize intracellular components such as nucleic acids and play an important role in viral sensing. TLRs have an extracellular leucine-rich repeat region and an intracellular domain that is shared with the interleukin-1 R, called a Toll-IL1R (TIR) domain. This domain interacts with downstream adaptors including MyD88, TIRAP/MAL, TRIF and TRAM to initiate signaling pathways leading to activation of NFkB and interferon responsive genes that coordinate the transcription of innate response genes essential for host defense. Emerging evidence supports a role for the inappropriate activation of TLR signaling in sterile inflammatory diseases such as rheumatoid arthritis, lupus and atherosclerosis [38-40]. Studies in hyperlipidemic mouse models have implicated TLR2, TLR4 and MyD88 in both endothelial and macrophage responses that promote atherosclerosis [41-43].

Toll-like receptors as targets of inflammatory signaling pathways

Under normal conditions, endothelial cells express low levels of TLRs where these receptors perform innate immune surveillance. However, expression of TLR1, TLR2, TLR4 and TLR6 is markedly upregulated by endothelial cells and macrophages in atherosclerotic arteries suggesting a role for these TLRs in the initiation and/or maintenance of vascular inflammation [44]. Targeted deletion of MyD88, a key signaling adaptor for all TLRs except TLR3, provided the first genetic evidence of a role for these receptors in atherogenesis [41,42]. $Myd88^{-/-}Apoe^{-/-}$ mice fed a Western diet for 8 weeks showed a dramatic decrease in atherosclerotic lesion area and macrophage content compared to $Apoe^{-/-}$ controls. Gene expression profiling of the aortas of these mice revealed significant reductions in numerous chemokines, suggesting that TLR signaling pathways promote monocyte recruitment into the artery wall [41]. Subsequent studies have confirmed roles for TLR2 and TLR4 in atherogenesis [42,43], however the ligands that activate these signaling receptors remain ill-defined. Targeted deletion of TLR2 in the $Ldlr^{-/-}$ mouse model led to a 50% reduction in lesion size in both the aortic sinus and the aorta of mice fed a Western diet [43]. Interestingly, transplantation studies revealed that cells of bone marrow origin did not account for the effect of TLR2 on atherosclerosis, suggesting a pro-atherosclerotic role for TLR2 signaling in endothelial or potentially other vascular cells [43]. Analysis of TLR4 deficiency Apoe^{-/-} mice also showed significant benefit, with 25% and 55% reductions in atherosclerotic lesion area in the aorta and aortic sinus respectively [42]. To date, the cell types responsible for this effect have not been delineated, however, modified forms of LDL have been shown to activate TLR4 in both macrophages and endothelial cells [45-47].

Despite genetic evidence for a role of TLR2 and TLR4 in atherogenesis, the ligands that trigger these signaling pathways in vivo remain a matter of speculation. Studies have failed to show a requirement for microbial infection in atherogenesis, suggesting that disease is driven by endogenous TLR ligands. Numerous putative endogenous ligands present in atheroma have been suggested, including modified lipoproteins and their component lipids, hyaluronan, fibronectin extra domain A, high-mobility box chromosomal protein 1 (HMGB1), heat shock proteins and apoptotic cells, however the role of these ligands in activating TLR signaling in lesional cells awaits further study. Interestingly, recent evidence suggests cross-talk between the scavenger and Toll-like receptor pathways. CD36 has been shown to cooperate with the TLR heterodimer TLR2/TLR6 to specify the cytokine signaling response to *S. aureus* and its cell wall component lipoteichoic acid [29,30]. Additionally, combinatorial signaling via SR-A and TLR4 appears to regulate macrophage apoptosis under conditions of endoplasmic reticulum stress, such as that induced by cellular free cholesterol accumulation [48]. Thus, scavenger receptors may help to present ligands to the appropriate TLR or to fine-tune TLR signaling responses.

As recognition of the role of TLRs in promoting chronic inflammatory conditions grows, significant efforts have focused on developing antagonists of TLR signaling pathways involved in atherosclerosis, as well as systemic lupus erythromatosis, rheumatoid arthritis and inflammatory bowel disease. Numerous vaccines and TLR antagonists directed against pathogen intervention are in clinical development (summarized in [49]), however, their utility in combating sterile inflammatory diseases is unknown. Given the roles of TLRs in host defense against invading microorganisms, it is unclear whether these pattern recognition receptors will be useful therapeutic targets for such chronic diseases. Moreover as TLR pathways, like scavenger receptor, have also been implicated in homeostatic repair processes that may be essential to atherosclerotic lesion regression, their targeting may prove to be a double-edged sword. Finally, should the TLR antagonists prove useful in inhibiting chronic inflammatory diseases, it seems highly likely that the nucleotide-binding oligomerization domain-like receptor family (NLR) of proteins, which interact with TLRs to signal through a complex called the inflammasome, will also be tapped for potential new therapeutic targets [50]. Specific targeting of NLRs in atherosclerosis has not been reported to date.

Therapeutic challenges in targeting innate immune pathways for atherosclerosis

While the evidence implicating the activation of innate immunity pathways in atherosclerosis is strong, the challenges of creating new therapeutics directed at these pathways are formidable. The role of innate immunity in protecting against microbial pathogens raises the specter of rendering hosts susceptible to a multitude of infectious agents when these pathways are interrupted for therapeutic benefit. Many of the mice that have been genetically engineered to lack components of the innate immune system are capable of surviving into adulthood without apparent major infection susceptibility, but when they are challenged with specific infectious agents, they are clearly impaired in their host defense mechanisms. Any attempt to weaken these defense systems for therapeutic benefit would, therefore, have to carefully examine the issue of infection susceptibility in humans. The widespread clinical use of TNF- α inhibitors, glucocorticoids, and other suppressors of inflammatory responses for the treatment of arthritis demonstrates that reductions in chronic inflammation can be targeted with acceptable infection risk. The highly redundant nature of the host defense response system makes it likely that the innate immune pathways could also be safely modulated for therapeutic gain and the many TLR antagonists marching toward clinical application for non-atherosclerosis chronic inflammatory conditions makes it inevitable that this hypothesis will be tested.

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A more difficult challenge in creating anti-inflammatory treatments for atherosclerosis centers on the practical difficulties of testing their efficacy, as our current animal models cannot be relied on to predict human clinical outcomes. Differences in lipoprotein metabolism, arterial size and composition, and mediators of inflammation all dictate caution in extrapolating benefits in murine models to likely outcomes in people. Unlike treatments directed at lowering LDL cholesterol, which is currently accepted as a surrogate endpoint by the FDA, atherosclerosis treatments directed at targets other than LDL will require human clinical trials to determine if a therapy confers benefit. The recent experience with the HDL-raising drug torcetrapib illustrates the complexity and enormous cost of such an undertaking. The hope that shorter trials with fewer patients, all imaged by coronary intravascular and carotid ultrasound techniques, might provide a cost-effective mechanism to evaluate an unproven new drug's therapeutic efficacy was being tested in torcetrapib's development program. The drug's failure to confer a benefit on cardiovascular outcomes, perhaps because of aldosterone-mediated offtarget effects on blood pressure and cardiac function, means that we will have to wait for a different compound or class to establish this new drug development paradigm [51]. While neither the coronary IVUS or carotid studies of torcetrapib demonstrated a benefit of the drug on atherosclerosis, consistent with its lack of benefit in the independent clinical outcomes trial, they also did not demonstrate a clear worsening of atherosclerosis in their primary endpoints [52-54]. As a substantial percentage of the increased CV morbidity in the torcetrapib trial was not clearly atherosclerosis-mediated, it is possible that these ultrasound methods did accurately capture the effect on atherosclerosis and that they therefore will prove extremely valuable in future treatment trials. Nevertheless, the bar remains high for establishing the value of non-LDL, anti-atherosclerosis therapies and this is a significant impediment to their clinical development. Much more effort is needed in the field to improve animal models, enhance imaging methods that are directed at evaluating mechanisms of inflammation in atherosclerosis (such as monocyte/macrophage tracking in and out of artery walls), and improve our assessment of the complex structure of atheromata. These advances will likely require consortial efforts between academia and the imaging and pharmaceutical industries, using collaborative arrangements that carve out domains of pre-competitive, mutual interest.

Conclusion

A growing body of basic science has implicated innate immunity pathways in the development and progression of atherosclerosis. These pathways result in the establishment of a sterile, chronic inflammation in the artery wall that ultimately leads to the narrowing of the arterial lumen and the subsequent rupture of plaques, which are the critical pathophysiological underpinnings of clinical coronary artery disease events. While our knowledge of the receptors involved in innate immunity and their downstream signal transduction pathways has expanded rapidly in the past decade, the precise molecular structures that trigger their engagement in atherosclerosis remain undefined. Drugs are currently in development that could inhibit several of the innate immune pathways, but due to the complexity and cost of evaluating atherosclerosis treatment effects in humans, these molecules are will enter the clinic as treatments for other chronic inflammatory diseases first. Perhaps via this secondary route, we will get our initial glimpse into the role of such drugs on human atherosclerosis. In the meantime, progress in the development of more predictive animal models of atherosclerosis and the functional imaging of atherosclerotic lesions is urgently needed, if these innate immunity pathways are to be targeted directly for the treatment of the major cause of human morbidity and mortality in the developed world.

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Figure 1.



Figure 2.