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CONTEMPORARY REVIEW

"How to Release or Not Release, That Is the Question." A Review of Interleukin-1 Cellular Release Mechanisms in Vascular Inflammation

Evan Kidder, BS; Siddhartha Gangopadhyay, PhD; Sheila Francis, PhD; Mabruka Alfaidi, MD, PhD

ABSTRACT: Cardiovascular disease remains the leading cause of death worldwide, characterized by atherosclerotic activity within large and medium-sized arteries. Inflammation has been shown to be a primary driver of atherosclerotic plaque formation, with interleukin-1 (IL-1) having a principal role. This review focuses on the current state of knowledge of molecular mechanisms of IL-1 release from cells in atherosclerotic plaques. A more in-depth understanding of the process of IL-1's release into the vascular environment is necessary for the treatment of inflammatory disease processes, as the current selection of medicines being used primarily target IL-1 after it has been released. IL-1 is secreted by several heterogenous mechanisms, some of which are cell type–specific and could provide further specialized targets for therapeutic intervention. A major unmet challenge is to understand the mechanism before and leading to IL-1 release, especially by cells in atherosclerotic plaques, including endothelial cells, vascular smooth muscle cells, and macrophages. Data so far indicate a heterogeneity of IL-1 release mechanisms that vary according to cell type and are stimulus-dependent. Unraveling this complexity may reveal new targets to block excess vascular inflammation.

Key Words: atherosclerosis ■ inflammasome ■ interleukin-1 ■ release mechanisms ■ therapeutic options ■ vascular inflammation ■ vascular wall cells

Cardiovascular disease is the leading cause of disease death worldwide[1] and is characterized by the underlying disease process of atherosclerosis, a narrowing or hardening of large and medium-sized arteries.[2] Despite many advances in disease management, therapies are still insufficient to fully mitigate atherosclerosis, which consequently affects cardiovascular disease risk and mortality.[2] Atherosclerosis was classically thought of as a disease characterized solely by glucose and lipid metabolism pathologies.[3] However, several observational studies led by Ridker, Libby, Abbate, and others documented an increase in plasma proinflammatory mediators, namely CRP (C-reactive protein), a surrogate marker for IL (interleukin)-1, as a major inflammatory culprit in atherosclerosis, in patients with coronary events.[4–6] These findings and subsequent experimental studies suggest that inflammation plays a critical role in the pathophysiology of atherosclerosis, presenting novel and unique opportunities for targeting cardiovascular disease in patient care.

In atherosclerosis, many cytokines are released locally in the vessel wall such as IL-1, IL-18, and IL-6,[7] with IL-1 having been extensively investigated in animals and humans,[8] and have been shown to be reduced using the traditional lipid-lowering medications (eg, atorvastatin).[9] Within atherosclerotic plaques, IL-1 can be released from endothelial cells (ECs),[10] vascular

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Nonstandard Abbreviations and Acronyms

EC	endothelial cell
MyD88	myeloid differentiation primary response protein 88
NF-κB	nuclear factor-kappa B
NLRP3	nucleotide-binding oligomerization domain, leucine-rich repeat family pyrin domain-containing 3
VSMC	vascular smooth muscle cell

smooth muscle cells (VSMCs),[11] and immune-derived cells[12] through a stimulus dependent and cell-type specific release process.[13] Once released, IL-1 plays a pivotal role in atherosclerosis progression from early stages of atherogenesis[14] to the formation of complex unstable lesions.[15] Recently, we have shown a role for endothelial IL-1 in linking the disturbed atherosclerotic environment to endothelial-to-mesenchymal transition and plaque instability.[16]

The term IL-1 refers to closely related genes on chromosome 2 that code for 2 functionally different proteins, IL-1α (IL-1 alpha) and IL-1β (IL-1 beta).[17] Polymorphisms in IL-1β (rs1143634) and IL-1α (rs1800587) have been linked to the increased inflammation and coronary artery disease.[18] IL-1α is constitutively produced in all mesenchymal cells and can be secreted by necrotizing cells in an already active precursor form.[19] Upon its secretion, it binds to the plasma membrane and acts locally to prime inflammasome activation.[19] By contrast, IL-1β (the main circulating form of IL-1[20]), is first produced as an inactive pro-form (pro-IL-1β) and is released from cells as active mature (21- to 17-kDa) forms.[21] IL-1 is a leaderless cytokine that lacks the signal peptide responsible for directing secretory proteins through the endoplasmic reticulum-Golgi pathway of protein secretion.[22] The proposed mechanisms by which IL-1 is released, such as lysosome-regulated secretory routes,[23] microvesicle shedding,[24] exosomal mediated release,[25] and potentially via specific channels/pores,[26] are summarized in the next sections and in Figure 1. This review discusses IL-1β's continually emerging role in vascular inflammation and the proposed routes of secretion of IL-1 from different vascular wall cells.

IL-1 ISOFORMS INTERACT WITH THE IL-1RI/II RECEPTOR

Once released, both IL-1α and IL-1β bind to the same receptors, including IL-1RI or IL-1R1 (IL-1 receptor type I) and IL-1RII or IL-1R2 (IL-1 receptor type II)[17] (Figures 2 and 3). An important component of IL-1R signal activation is the interaction between IL-1 and IL-1R AcP (IL-1R accessory protein).[27] IL-1R AcP is also called IL-1R3 and it can act as a coreceptor to the other members of the IL-1 family, such as IL-33 (fully reviewed in Dinarello[17]). Binding of IL-1 to IL-1R AcP leads to recruitment and binding of intracellular adaptor proteins, including MyD88 (myeloid differentiation primary response protein 88).[28] MyD88 binds to the intracellular domain of IL-1R1 and triggers cascade of kinases that ultimately promotes nuclear factor-kappa B (NF-κB) activation.[29] In addition to IL-1R1, it has been reported that MyD88 also binds to other receptors such IL-18R and TLR2/4 (toll-like receptors 2/4).[17]

IL-1 is a proinflammatory cytokine, and for it to be kept at negligible circulatory levels in a disease-free state, the production of IL-1 is controlled by multiple autoregulatory mechanisms. A naturally occurring competitive inhibitor, IL-1ra (IL-1 receptor antagonist), competes with IL-1 binding at the receptor level without downstream signaling effects[30] due to a lack of engagement with the IL-1R AcP interacting domain.[20] Further control is provided by a decoy receptor, IL-1RII or IL-1R2, which binds to IL-1 without causing any significant changes in cellular system.[17] IL-1R2 binds to IL-1, limiting IL-1–IL-1R1 interactions[17] (Figure 3). Studies on IL-1R2 are limited and the functional roles of this receptor in atherosclerosis development and progression are still unclear. In contrast, there has been sustained interest in the role of IL-1ra (anakinra) as an antiatherogenic drug that has been shown to reduce atherosclerotic plaque sizes and downregulate inflammatory cytokines (IL-1β and IL-6) in vitro.[31]

PROCESSING OF THE IL-1 PRECURSOR

We and others have shown increased levels of IL-1β mRNA and protein in human coronary atherosclerotic plaques.[8,32] Found mainly in ECs, VSMCs, and macrophages, these increased levels provide compelling evidence that IL-1β is synthesized and released locally in the arterial wall during the development of atherosclerosis. In the field of IL-1 release, most studies have been conducted on monocytes and macrophages and the importance of these cells in the pathophysiology of atherosclerosis and inflammatory vascular pathology is well known and IL-1 release mechanisms have been well documented.[33] In contrast, this review focuses on the current understanding of IL-1 release mechanism(s) in the vascular wall cells.

In response to immune stimulation, ligand engagement of IL-1R1 or TLRs causes MyDDosome complex aggregation and activation through the intracellular domains of IL-1R1, linking ligand stimulation to the activation of both IRAK4 (IL-1–activated kinase type 4)

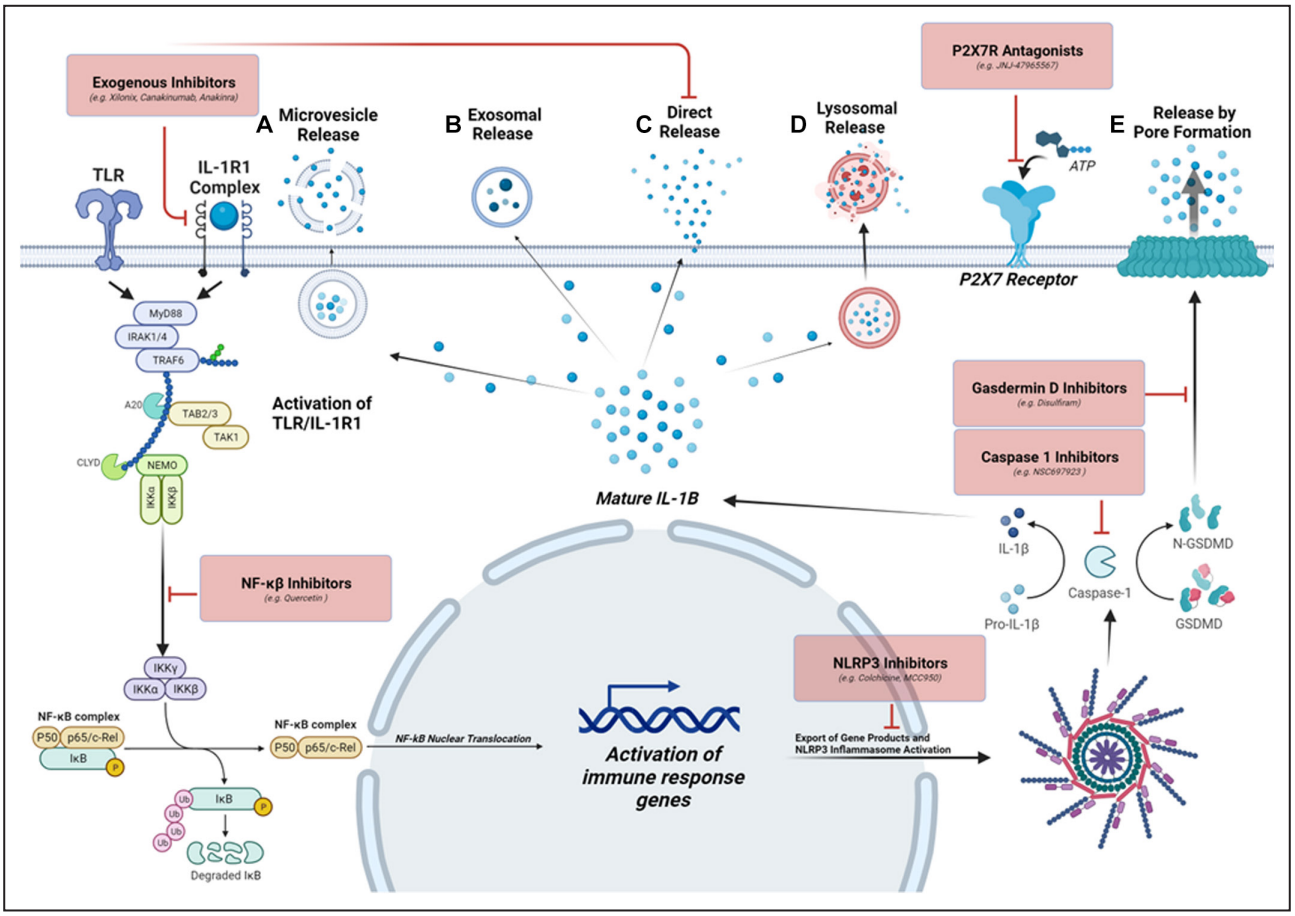

Figure 1. The molecular mechanisms of IL-1 release.
IL-1 binding to IL-1R1 results in a cascade of activation and phosphorylation, which ultimately promotes NF-κB nuclear translocation and *transcription* and formation of pro-IL-1β. After the generation of pro-IL-1β (the inactive form of IL-1β: 31 kDa), the inflammasome complex is assembled in response to a second stimulus, such as ATP activation of P2X7 receptors. Following this, the inflammasome complex is assembled, and procaspase-1 is cleaved into active caspase-1, which cleaves pro-IL-1 into various forms including the mature 17-kDa IL-1β. IL-1β can be released as a soluble (membrane-unbound/ free) or a vesicular (membrane-bound) form. Throughout the decades of research on IL-1β, different mechanisms for mature IL-1β release have been proposed, suggesting that IL-1 secretion is not a unified mechanism but rather the release varies according to cell type and is context- and stimulus-dependent. The proposed mechanisms for soluble IL-1 secretion are: microvesicular shedding mechanisms (**A**); exocytosis pathways of multivesicular bodies and /or exosome release (B); direct IL-1 release through a hyperpermeable plasma membrane (**C**); IL-1 release by lysosomal exocytosis (**D**): following caspase-1 activation, secretory lysosomes fuse to the plasma membrane and release their IL-1β contents by a process of exocytosis, release by gasdermin D or other pore (**E**). A combination of release mechanisms could occur in various repair or pathological settings. Boxes have also been depicted in this figure to show sites in the mechanistic release process of IL-1 that would be affected by therapeutic intervention. Therapeutics mentioned are further expounded upon in the Table. GSDMD indicates gasdermin D; IL-1β, interleukin-1 beta; IL-1R1, interleukin-1 receptor type-I; IL-1Ra, IL-1 receptor antagonist; IKKB, I-kappa-B kinase B; IRAK1/4, interleukin-1 receptor-associated kinase 1/4; NF-κB, nuclear factor-kappa B; NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat family pyrin domain-containing 3; and TLR, toll-like receptor.

and IRAK1 and subsequent IKKB (I-kappa-B kinase B) phosphorylation and NF-κB activation (Figure 2), leading to the production and accumulation of inactive pro-IL-1β (31-kDa) in the cytoplasm.[34] Along with pro-IL-1β transcription stimulation, similar cytokines within the IL-1 family such as IL-18 can also be produced and thence processed by caspase-1 similar to pro-1L-1β and some other caspase independent methods.[17]

In addition to caspase-1, other cysteine proteases such as caspase-4 and caspase-5 have been implicated in IL-1 release. Of note, caspase-4 is found in humans and not in mice and transgenic mice expressing caspase-4 exhibited significantly higher IL-1 and IL-18 secretion from primed macrophages by caspase-1–mediated activation. Caspase-5 can induce IL-1 secretion in lipopolysaccharide-primed monocytes, without the need to activate caspase-1[35]. Additionally, murine caspase-11 (a member of caspase-1 subfamily and most homologous to human caspase-4) has been shown to be induced by lipopolysaccharide activation and does not directly process pro-IL-1 but activates caspase-1 to simulate the processing (functional

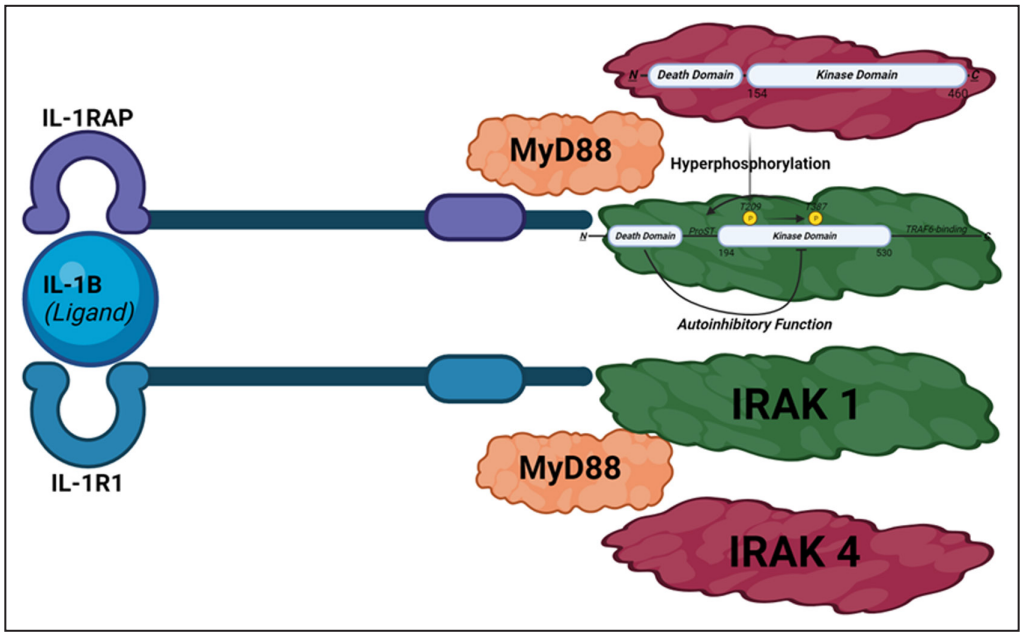

Figure 2. The IL-1 signaling pathway.
IL-1 binding to its receptor IL-1R1 and to IL-1R AcP results in a downstream activation that eventually leads to IRAK4 and IRAK1 recruitment in order to bind to the adaptor protein MyD88. A series of IRAK4/1 phosphorylation and activation at multiple sites subsequently leads to IRAK1 dissociation from the receptor complex, leading to signaling cascades that induce transcription of proinflammatory genes. IL-1β indicates interleukin-1 beta; IL-1R1, interleukin-1 receptor type-I; IL-1R AcP, IL-1 receptor accessory protein; IRAK1/4, interleukin-1 receptor-associated kinase 1/4; and MyD88, myeloid differentiation primary response protein 88.

differences between caspase-4/5/11 reviewed by Matikainen et al[36]). Further studies are needed to dissect the exclusive and overlapping roles between these proteases as well as their involvement in the canonical caspase 1/inflammasome pathway. Moreover, in vascular cells (ECs and VSMCs) the role of all of the caspases is still underinvestigated, especially in the context of atherosclerosis and local IL-1 release.

The term inflammasome denotes the collection of several molecules assembled in response to a secondary inflammatory stimulus subsequently causing protein maturation.[37] The NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat family pyrin domain-containing 3) inflammasome is a multimeric complex containing 3 components: NLRP3, ASC (apoptosis associated speck-like protein containing a caspase recruitment domain), and procaspase-1. The activation of NLRP3 inflammasome is tightly controlled and activation and cleavage of procaspase-1 into active caspase-1 usually occurs in response to a second hit stimulus and before the release of active/mature IL-1β (ie, P2X7R [P2X7 receptor] activation by ATP).[38]

Cells in the vascular environment respond to inflammatory stimuli by a variety of other inflammasome mechanisms leading to heterogeneity in responses temporally and locally. NLRP3- and AIM2-directed mechanisms have been detected in monocyte/macrophage intimal infiltrates in early atherosclerotic plaque progression.[37] Additionally, NLRP1 inflammasome activity has also been shown to drive endothelial cell inflammatory largely in in peripheral artery disease.[37] Additionally, caspases also are activated by a variety of other mechanisms within the vascular environment. Caspase-1 and caspase-5 expression (along with NLRP3 and AIM2) are overwhelmingly expressed in lymphoid infiltrates of the media and adventitia of the aorta.[37] A comprehensive review of inflammasome pathophysiology in vascular pathology may be found in[37]. Classically, however, the pro-form of IL-1 (31-kDa) in immune cells (and vascular cells) is cleaved to a mature biologically active form (17-kDa) by caspase-1, a component of the inflammasome.[38,39] The inflammasome principally referred to herein is the NLRP3 inflammasome, which is predominately activated with the vascular environment (VSMCs, monocytes/macrophages, ECs; Figure 1).

In addition to caspase-1, the IL-1β pro-form can be cleaved by other proteases, such as neutrophil elastase, cathepsins, serpins.[40] These proteases likely found in the vessel wall cleave pro-IL-1β into a biologically active IL-1β at distinct sites to that of caspase-1, which results in the production of (18- to 21-kDa) isoforms of active IL-1β.[10,41] Inside the vascular wall cells, the site of IL-1 processing is largely unknown. Moreover, systematic

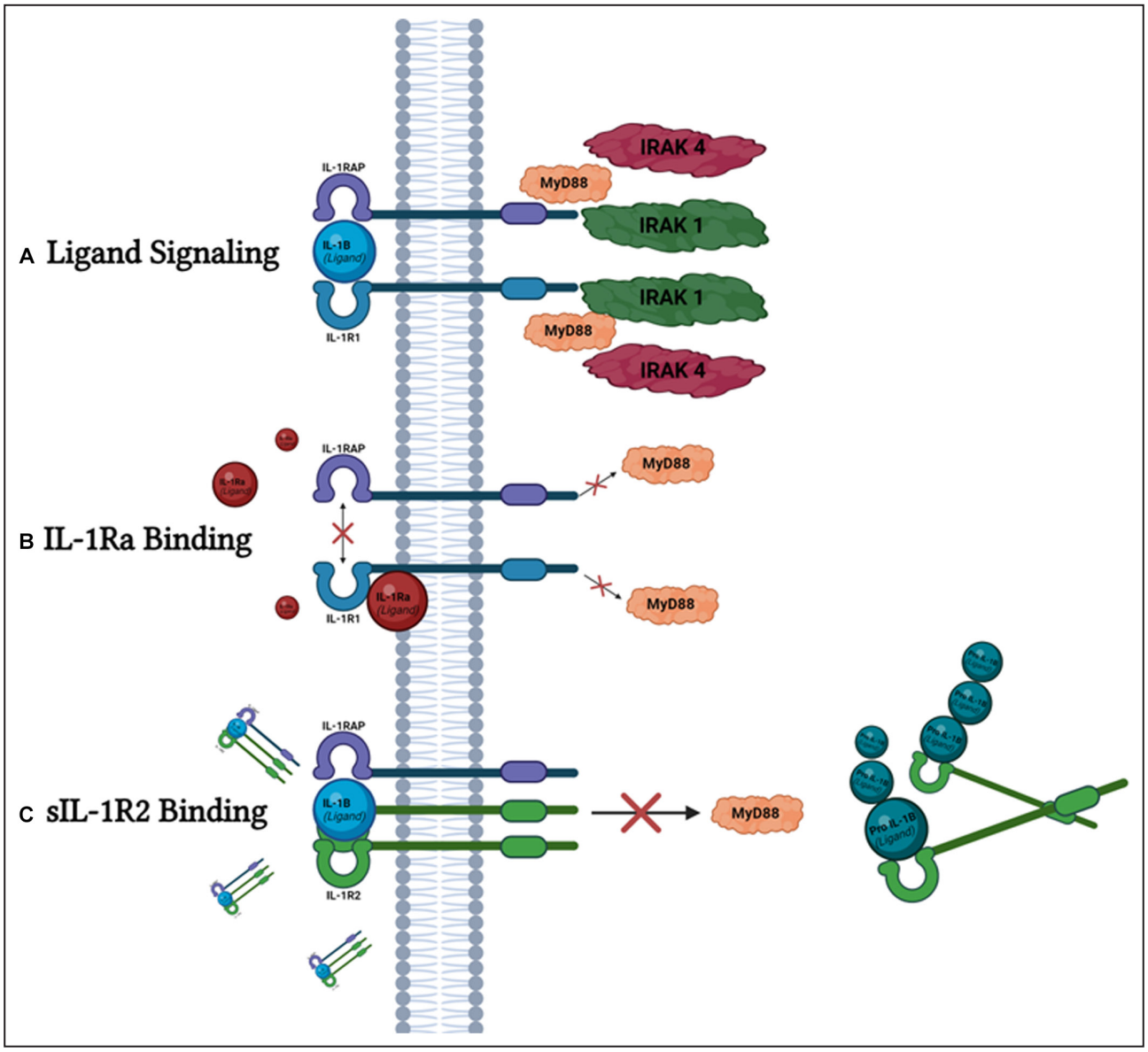

Figure 3. Antagonism of IL-1 signaling pathway.
Classical IL-1a/IL-1B signaling through the IL-1R1 complex as described in Figure 2 (**A**). IL-1Ra is a natural competitive inhibitor that competes with the IL-1 ligand at the receptor site (**B**). Once it binds to the receptor, there is no ensuing downstream activation, because IL-1Ra lacks the domain that allows it to bind to the IL-1R AcP. Binding between IL-1 and IL-1R2 does not initiate the activation pathway (**C**). IL-1R2 molecules are decoy receptors; once they bind to IL-1 they form a trimeric complex with IL-1R AcP, separate from the cells and exist in a soluble state (sIL-1R2). It is believed that by binding to IL-1R2, IL-1 is kept away from its signaling receptor (IL-1R1). sIL-1R2 can also bind to the pro-form of IL-1 (pro-IL-1) and possibly other forms, preventing cleavage by extracellular proteases. IL-1β indicates interleukin-1 beta; IL-1R1, interleukin-1 receptor type-I; IL-1R AcP, IL-1 receptor accessory protein; IL-1Ra, interleukin-1 receptor antagonist; IRAK1/4, interleukin-1 receptor-associated kinase 1/4; MyD88, myeloid differentiation primary response protein 88; and sIL-1R2, soluble interleukin-1 receptor type-2.

studies of specific cleavage mechanisms for pro-IL-1β in vascular cells including ECs and smooth muscle cells in normal and atherogenic disease settings are needed to further characterize IL-1β secretion. So far, it has been shown that there are caspase-1–independent pathways that cleave pro-IL-1 from studies carried out in caspase-1 knockout mice, where IL-1β was still detected in plasma in these settings.[42] A complication is that caspase expression is different in humans versus mice (mice lack caspase-4, but caspase-11 may substitute[43]). Future studies are needed to dissect the exclusive and overlapping roles of caspases and proteases in the cleavage of pro-IL-1β in mice and humans and in the vascular cells. Until this is done, the scale of the contribution of mature IL-1β and other isoforms in vascular disease processes is likely to be underestimated.

RELEASE OF IL-1β INTO THE EXTRACELLULAR ENVIRONMENT

The mechanisms for trafficking of IL-1β outside cells into the extracellular environment in vascular cells remain poorly understood. There is general consensus that in contrast to TNF-α (tumor necrosis factor alpha) or IL-6, which have a signal sequence in proximity to their N terminus directing their progression through the cell factory machinery.[44] IL-1β lacks this directional locator signal.[22] Other control signals clearly exist, as cellular IL-1β becomes ubiquitinated in inflammatory settings making it inaccessible to cleavage by caspases and directing it to the proteasomes.[45] Posttranscriptional control mechanisms may therefore regulate activity and release. Interestingly, if endoplasmic reticulum-Golgi trafficking inhibitors are used in vitro, IL-1β is still released,[46] raising a question as to exactly how, in certain settings, IL-1β gets into the extracellular space. Understanding of the mechanisms leading to the release of bioactive IL-1β may help identify new targets and improve our understanding of the inflammatory milieu present during atheroma progression.

IL-1 RELEASE IN THE CONTEXT OF VASCULAR INFLAMMATION

One of the first IL-1 release mechanisms proposed was cell lysis in response to physiological cell injury.[47] However, as research on mechanisms of cell death has become more refined, apoptosis, pyroptosis (a rapid and inflammatory form of cell stress/programmed cell death), and other regulated forms of cell death such as secretory autophagy,[48] were proposed to induce IL-1 release in specific cell types. Vascular cell data are limited, but in macrophages, apoptosis stimuli lead to caspase-8 dependent processing of IL-1β and release.[49] Extracellular ATP, acting as a damage-associated molecular pattern, can also promote IL-1β release through activation of P2X7R or through forming a membrane channel to promote the secretion process of IL-1.[50] In endothelial cells, however, the expression of P2X7R is relatively low and thus the release of IL-1β is inefficient,[51] demonstrating the differences between macrophages and endothelial cells in terms of mechanism of IL-1 release.

The potential complexity of the processing and the release of IL-1 was evident more than 20 years ago when microvesicular release of IL-1β from THP-1 monocytes was first described.[24] After activation (step 1), a second stimulus (step 2) of extracellular ATP lead to phosphatidyl flipping, P2X7R activation and rapid release of plasma microvesicles containing IL-1β within minutes.[24] A similar mechanism was described in other cells, such as mature dendritic cells where IL-1β was detected in microvesiclesonly from mature rather than immature dendritic cells despite vesicles from both cell types containing caspase-1, caspase-3, and cathepsin D[52]. Canonical secretion of IL-1β appears to be regulated by a 2-step process; signal 1 involves transcription of IL-1β and pro-IL-1β accumulation within cells. Signal 2 is mandatory for the release and consists of activation of the zymogen caspase-1 to enable processing of pro-IL-1β. For the very rapid release of IL-1β, caspase-1 also has been shown to activate gasdermin D pores,[26] causing the secretion of a soluble form of IL-1β (Figure 1).

In ECs derived from human coronary arteries, we were the first to show that in response to exogenous neutrophil elastase treatment, IL-1β is present in microvesicles without caspase-1 or any components of the inflammasome complex, suggesting that caspase-1/inflammasome is not always required for the vesicular IL-1 release.[10] Additional secretory routes for IL-1β are proposed to be via exocytosis of secretory lysosomes, via exosomes (lipid bilayer particles 30–150 nm) or via exomeres derived from multivesicular bodies or a combination of mechanisms of all these routes of secretion.[46] However, these routes are generally poorly understood in human vascular wall cells.

Under control healthy conditions, vascular cells do not produce IL-1; however, experimental studies have indicated that cultured ECs and VSMCs synthesize large amounts of IL-1β in response to different cytokine stimulations.[10] In addition to these cytokines, other atherosclerosis stimuli present in diseased vessel wall such as neutrophil proteases,[10] oxidized lipids, cholesterol crystals, and calcium phosphate particles, promote IL-1β synthesis and release. Indeed, as early as the early the 1990s, multiple lipid oxidation products were shown to induce release of IL-1β from human peripheral mononuclear cells; this was most pronounced with nontoxic low concentrations of oxidized low-density lipoprotein (LDL) for short amounts of time.[53] We have shown recently that for primed coronary artery ECs incubated for 6 hours with oxLDL, IL-1β was released via a nonvesicular canonical caspase-1, NLRP3-dependent mechanism.[54] Interestingly, primed VSMC treated with oxidized LDL (for just 2 hours) also released IL-1 to a similar extent, but through a caspase-1, non-NLRP3-dependent pathway.[54] This contrast with the mechanisms of IL-1β release was also shown by calcium phosphate deposits, which stimulate IL-1β release from human VSMCs by activation of SYK, caspase-1 cleavage, and release in part through exosomes.[55] Exosomes also seem to be responsible for IL-1β release in primed human coronary artery ECs after neutrophil elastase treatment.[10] Taking these observations together, the common part of the mechanism in vascular wall cells appears to be caspase-1 and this perhaps suggests that caspase-1 inhibitors could be helpful in vivo. Numerous challenges

Table 1. Current Therapeutic Strategies Targeting Either IL-1 Isoforms of or Mechanistic Steps in Its Transcription, Processing, and Subsequent Release

Drug name	Type of drug	Study cited	Target of drug	Type and population studied	Results
Quercetin	Flavonoid	Sul et al[88]	NF-κB inhibition	Human in vitro: human alveolar epithelial cells	In response to oxidative stress caused by lipopolysaccharides, quercetin significantly reduced *IL-1β* mRNA levels due to NF-κB inhibition.
Canakinumab	Monoclonal antibody	Ridker et al[89]	IL-1β antagonism	10 061 Patients with a history of a MI and a high CRP of ≥2 mg/L	Canakinumab showed a dose-dependent CRP reduction (26% in 50 mg; 37% in 150 mg; 41% in 300 mg). HRs: in the 50-mg group, 0.93 (95% CI, 0.80–1.07; *P*=0.30); in the 150-mg group, 0.85 (95% CI, 0.74–0.98; *P*=0.021); and in the 300-mg group, 0.86 (95% CI, 0.75–0.99; *P*=0.031)
Colchicine	Antimitotic drug	Samuel et al[90]	NLRP3 inhibition	4745 Patients 30 days after an MI, colchicine (N=2366)/placebo (N=2379)	Colchicine was shown to have a statistically significant effect against stroke (HR, 0.26 [95% CI, 0.10–0.70]) as well as a statistically significant effect against rehospitalization for angina leading to revascularization (HR, 0.50 [95% CI, 0.31–0.81]).
Xilonix	Monoclonal antibody	Hong et al[91]	IL-1α antagonism	Nonsmall cell lung cancer patients	Xilonix was shown to bind to IL-1α.
Anakinra	Recombinant IL-1Ra	Ku et al[31]	IL-1R1 blockade	Murine model, apoE$^{-/-}$ mice	Anakinra reduced plaque size 30.6% at 25 mg/kg dosage and 25.2% at the 50 mg/kg dosage. (*P*<0.05).
JNJ-47965567	Small molecule inhibitor	Mücke-Heim et al[92]	P2X7 receptor antagonist	In vitro human microglial cell cultures	JNJ-47965567 was shown to decrease proinflammatory phenotypical expression of cytokines (*P*<0.05).
NSC697923	Small molecule inhibitor	Cao et al.[93]	Caspase-1	Lipopolysaccharide-stimulated macrophages	NSC697923 was shown to prevent priming of the NLRP3 inflammasome by binding to the active pocket of caspase-1. Additional effects inhibiting NF-κB and gasdermin D pore formation were also shown.
Disulfiram	Cysteine-modifying drug	Hu et al[94]	Gasdermin D	Human THP-1 cells	Disulfiram was shown in THP-1 cells to block gasdermin D pore formation in cells undergoing pyroptosis thereby preventing the release of IL-1β.
OLT1177	Active moiety (β-sulfonyl nitrile)	Marchetti et al[95]	NLRP3 inflammasome inhibitor	Animal models of chronic inflammation, patients with HF	In vivo in animals, it has been shown to inhibit NLRP3 inflammasome activation. In a phase 1B clinical study, it has been shown to be safe and well-tolerated in patients with HF.
Tranilast	Antiallergic drug, analogue of a tryptophan metabolite	Chen et al[96]	Inhibits NLRP3 inflammasome assembly	Animal model of atherosclerosis	Tranilast blunted the initiation and progression of atherosclerosis.

Therapeutics are herein stratified by their target of inhibition or modulation and show the heterogeneity of strategies to target common pathways in the synthesis and release of IL-1. They are not to serve as a comprehensive list, but rather, this list is to serve as a companion to the aforementioned drug targets by providing specific examples of inhibitors in said classes. apoE indicates apolipoprotein E; CRP, C-reactive protein; HF, heart failure; HR, hazard ratio; IL-1β, interleukin-1 beta; IL-1α, interleukin-1 alpha; IL-1R1, interleukin-1 receptor type-I; IL-1Ra, IL-1 receptor antagonist; MI, myocardial infarction; NF-κB, nuclear factor-kappa B; and NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat family pyrin domain-containing 3.

have been identified in relation to caspase-1 inhibitors in clinical trials including a lack of specificity, efficacy, and some toxicity.[56] Moreover, IL-1β has also been shown to be cleaved to its active forms by non-caspase-1 dependent mechanisms, such as chymase, cathepsin G.[40]

IL-1 IS CRUCIAL IN EARLY ATHEROSCLEROSIS DEVELOPMENT

In atherosclerotic plaques, there is a mixed pattern of inflammatory process throughout the life course[57] and IL-1, chiefly IL-1β, has been shown to be critical in the development of early lesions.[58] Studies of atherogenic mouse strains highlight the importance of IL-1; for example, fatty streak lesions in apoE$^{-/-}$ (apolipoprotein E) knockout mice showed a significant decrease after IL-1ra administration.[59] Deletion of IL-1ra (*IL1RN*$^{-/-}$) in mice increased atherosclerosis[15] and overexpression of IL-1ra in transgenic mice with LDL receptor$^{-/-}$ or apoE$^{-/-}$ background showed a decrease in total lesion areas.[60] Individual IL-1 proteins have also been directly assessed and there is overwhelming evidence to suggest a critical role for IL-1β in early atherogenesis. Seminal work in the field on the genetic deletion of IL-1β in apoE$^{-/-}$ atherogenic mice resulted in a considerable reduction in lesion size, associated with a lowering of adhesion molecules and MCP-1 (monocyte chemoattractant protein-1).[14]

In terms of the key pathways, IL-1β enhances VSMC migration and proliferation by different mechanisms. IL-1β promotes the proliferation through platelet-derived growth factor BB[61] by inhibiting the expression of p21,[62] and it also enhances cell migration by NOTCH-mediated MMP (matrix metalloproteases)-2/9.[63]

Emerging evidence of the direct effects of IL-1α on atherogenesis has also been reported. Mice on a C57BL6/J background and deficient in IL-1α showed fewer fatty streaks after atherogenic diet feeding,[64] and it has been suggested that IL-1α promotes EC senescence[65] and VSMC-induced proinflammatory mediator production within the arterial wall,[66] confirming the importance of IL-1α in vascular wall inflammation. Collectively, these mechanisms and their effects are fundamental in triggering and promoting the development of atherosclerosis.

IL-1 INVOLVEMENT IN ADVANCED ATHEROSCLEROSIS IS COMPLEX AND NUANCED

The roles of IL-1, especially IL-1β, in advanced atherosclerosis have been shown to have opposing functions in laboratory studies. High levels of IL-1β have been linked with an increase in the number of infiltrating macrophages,[15] and IL-1 plays a significant role in the formation of advanced lesions in response to high-fat diet feeding in mice.[67] Excessive IL-1-induced proteolysis in atheroma associates with the risk of rupture,[68] a more nuanced role for IL-1 signaling in advanced atherosclerosis has been proposed. Mice lacking the ability to signal via IL-1R1 or where IL-1β is neutralized more readily acquire rupture-prone characteristics; however, it should be noted that these athero-prone characteristics do not automatically translate to a plaque rupture effect and are made based on plaque phenotype rather than actual ruptured plaques causing myocardial infarction. Of note, and a limitation of most mouse models of atherosclerosis, is that these do not present with classical plaque rupture as in humans.[69]

IL-1β seems to directly modulate VSMC phenotypes more than IL-1α, and these effects in advanced lesions seem to be different from those seen in early lesions.[69] In addition, IL-1β downregulates VSMC contractile markers through a NF-κB-mediated mechanism and this is independent of platelet-derived growth factor.[70] In terms of cell-type specific inhibition of IL-1 signaling, mice that specifically lacked IL-1R1 in VSMCs had more atherosclerosis and fewer VSMCs than their littermates,[71] whereas mice deficient in IL-1R1 in macrophages developed a favorable inhibitory response on atherosclerotic plaques with stable characteristics.[67] Data on inhibition of IL-1 signaling in endothelial cells in the context of atherosclerosis are still awaited.

Moreover, the VSMC effects of IL-1 could be potentially complicated by other mechanisms such as clonal hematopoiesis of indeterminate potential,[72] plaque erosion,[73] neutrophil extracellular traps formation,[74] and endothelial-to-mesenchymal transition.[75] Clonal hematopoiesis of indeterminate potential, increased by biological aging, is an expansion of hematopoietic clones carrying recurrent somatic, most frequently loss-of-function, alleles in the genes *DNMT32A*, *TET2*, *ASXL1*, *JAK2*, *PPM1D*, and *TP53* (fully reviewed in Amoros-Perez and Fuster[76]). *Tet2*$^{-/-}$ bone marrow transferred to LDL receptor$^{-/-}$ mice fed a Western diet led to accelerated atherosclerosis,[72] and this was associated with excessive IL-1β production,[77] which promoted P-selectin expression and endothelial activation, leading to increased macrophage infiltration and plaque growth.[77] These data support a role for IL-1β in the setting of accelerated atherosclerosis associated with *Tet2* loss-of-function.[78]

Proinflammatory mediators such as IL-1β released by infiltrating cells and vascular cells create environmental changes such as extracellular matrix degradation within artery walls promoting plaque instability or plaque rupture.[68] A new type of arterial wall damage called plaque erosion has been shown to contribute to symptoms in up to one third of patients with acute coronary syndrome.[73] With an unclear molecular mechanism, the endothelial layer on eroded plaques

is usually absent and they are usually less inflamed than ruptured plaques (reviewed in[79]). One proposed mechanism is that ECs exposed to blood flow develop distinct phenotypic changes and undergo apoptosis,[80] and this results in plaque erosion.[81] Also likely to feature is neutrophil extracellular traps formation, a process where web-like structures composed of granules, decomposed chromatin, and cytosolic proteins are released by neutrophils in response to pathogens.[82] High numbers of neutrophils and significant neutrophil extracellular traps formation have been detected in erosion-prone plaques in humans,[83] and interactions between neutrophils and ECs can alter many endothelial functions,[84] including production of IL-8 and IL-1β (reviewed fully in[74]).

In human tissue samples, pathological analysis of subendothelial cells shows that VSMCs- and fibroblasts-like features are associated with clinical presentation.[85] These subendothelial cells express the mesenchymal markers (Snail1, SLUG, TWIST1) and expression of endothelial markers (CD31, CD144) is reduced.[86] Additionally, these cells have fibroproliferative features, including propensity for extracellular matrix deposition, migration, and proliferation. Lineage tracing mice experiments have demonstrated that these cells are endothelial cells in origin, and thus, they are commonly referred to in the field as having undergone endothelial-to-mesenchymal transition (fully reviewed in[87]).

THERAPEUTIC OPTIONS MODULATING IL-1β BEFORE OR AFTER THE RELEASE?

IL-1 is a leaderless cytokine responsible for the vascular inflammatory response to injury and is implicated in several different processes be that of its induction or its release. This heterogeneity of activities and circumstances provides several unique targeting opportunities to develop modalities to modulate the IL-1 release from cells.

The IL-1R1 receptor is expressed in part as a response to damage-associated molecular patterns or pathogen-associated molecular patterns activating a cascade of actions that result in the maturation of IL-1β. This presents an interesting therapy modality as lowering the aggravating factors present in serum may lead to decreased expression and lower subsequent release of IL-1β. There are several medications addressing this stage of intervention, statin medications being a prime example by lowering cholesterol, cholesterol species and at the same time exerting anti-inflammatory effects through the IL-1 receptor.[9] Beyond the receptor complex, several treatment options exist in either inhibiting or disrupting the activation of NF-κB (Table),[88–96] which localizes to the nucleus to influence the transcription of mRNA leading to the NLRP3 inflammasome and pro-IL-1β production.

The NLRP3 inflammasome itself may be targeted by either disrupting its assembly or destroying its components. Direct inhibition of caspase-1 has similarly been shown to blunt the maturation of IL-1β. Direct inhibition of gasdermin D pore formation with compounds such as disulfiram have additionally been shown to inhibit the release of IL-1β by pore formation although not inhibiting the maturation of IL-1β.[94] Recently, colchicine specifically has also been shown to be an inhibitor of the NLRP3 inflammasome[97] and has been approved by US Food and Drug Administration as anti-inflammatory drug in patients with chronic coronary artery disease.[97] These and other representative compound/drug examples are described further in the Table.

Specific targeting of serum IL-1β is proven to be efficacious, especially with the monoclonal antibody canakinumab.[89] The CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) trial showed that this monoclonal antibody targeting IL-1β led to a marked decrease in CRP levels in a dosage-dependent level that reduced the incidence of negative events in this population studied.[89] Rilonacept is a soluble version of the IL-1R1 that has been shown to bind the different isoforms of IL-1 and reduce the levels of IL-1β in serum.[98] Xilonix neutralizes IL-1α[91] once it is released outside of cells. An additional successful approach has been tested to occupy IL-1 binding receptors on the cell surface (anakinra).[20] Anakinra specifically targets the effects of IL-1β in the extracellular environment by binding to and competitively blocking the IL-1R1 receptor.[31] In murine studies, anakinra has been shown to significantly reduce atherosclerotic plaque size and suppresses proinflammatory cytokines IL-1 and IL-6.[31]

The importance of understanding mechanistic effects leading to the reduction of IL-1 release is important as targeting efforts could be directed either at physical changes in the cells, for example, formation of pores or to the specific cleavage or trafficking of IL-1 inside cells. An example is of gasdermin D pore inhibition, specifically with compounds such as disulfiram, which mainly inhibit the process of cellular pyroptosis rather than broader IL-1β release and have no effect on the maturational process of IL-1β.[94] Thereby, surrogate measures of IL-1β in the exogenous environment may show a reduced presence, yet the mature ligand could still be contained within vascular cells and could be released upon another on-demand and discrete stimulus. As inflammatory disease processes are further characterized and studied, identification of the specific mechanisms of IL-1 release will help to pair a fitting therapeutic option with broader based approaches if required.

IL-1 is an excellent therapeutic target, but several cautionary effects persist. When using immune

modulating therapies, there exists a clear and inherent risk of sepsis resultant from immune suppression due to treatment.[89] Although it has been shown that this can be combated by coadministering antibiotics,[89] another risk profile is introduced. IL-1 is crucial in activation of the innate immune response and its integrity for specific cell signaling and an individual patient's response to novel infections is paramount. This knowledge highlights the importance of developing specific inhibitory therapeutics that are not agnostic of cell type. It is also critically important to consider that successful therapeutic intervention in inflammation driven diseases is characterized by early treatment, and the emerging evidence suggesting a sex-specific risk stratification for administering anti-inflammatories such as anakinra in the context of ST-segment–elevation myocardial infarction.[99] As new therapies based on release mechanisms emerge, these could also be involved in release of other leaderless cytokines, some of which could be helpful rather than detrimental to the healing process. Despite these concerns, IL-1 remains a clear and promising target in the field of cardiology and immune-related medicine, and the answer to modulating and correcting high serum amounts of IL-1 may reside in using an amalgam of timed treatments rather than using 1 modality for a long period at a high dose.

CONCLUSIONS AND FUTURE DIRECTIONS

With cardiovascular disease being the highest cause of mortality worldwide, the importance of the molecular understanding of disease processes such as atherosclerosis is paramount. Atherosclerosis recently has been shown to be mediated in large part by a disproportional inflammatory response that results in plaque formation and progression to an unstable phenotype. There is still much more to do to explore the release of IL-1 in particular settings. This includes investigation of other stimuli such as lipoprotein(a), oxidized LDL, and other forms of LDL, ceramides, amyloid, nucleic acids, and microparticles, and in pathologies relevant to vessel walls, endothelial-mesenchymal transition, plaque erosion, and cerebrovascular inflammation. In addition, the role of current and emerging therapies for coronary disease on the release of IL-1β in vessel walls is unknown. It is worth keeping in mind that inflammation driven by IL-1 in vessel walls consistently links dyslipidemia and risk factors to atherogenesis. Several existing therapeutic strategies target the canonical pathway of IL-1 release, but there is a current need for interventions targeting microvesicular release or release directed from channels or pores, while maintaining homeostatic immune functions. Despite the present need for further therapeutic options, current inhibitors and antagonists against specific steps in IL-1 release do provide options for stratification and specificity in targeting disease processes, and with further research, these may prove to be highly mechanistically specific. These questions and others raised from the current state of knowledge of IL-1 release in vascular environments may inspire future needed investigations into the heterogenetic nature of molecular release mechanisms of this principal leaderless cytokine.

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Disclosures

None.

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