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Interleukin-1 Beta as a Target for Atherosclerosis Therapy: The Biological Basis of CANTOS and Beyond

Peter Libby, MD

Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Abstract

Inflammatory pathways drive atherogenesis and link traditional risk factors to atherosclerosis and its complications. One inflammatory mediator has come to the fore as a therapeutic target in cardiovascular disease. The experimental and clinical evidence reviewed here support interleukin-1 beta (IL-1 β) as both a local vascular and systemic contributor in this regard. Intrinsic vascular wall cells and lesional leukocytes alike can produce this cytokine. Local stimuli in the plaque favor the generation of active IL-1 β through the action of a molecular assembly known as the inflammasome. Clinically applicable interventions that interfere with IL-1 action can improve cardiovascular outcomes, ushering in a new era of anti-inflammatory therapies for atherosclerosis. The translational path described here illustrates how advances in basic vascular biology may transform therapy. Biomarker-directed application of anti-inflammatory interventions promises to help us achieve a more precise and personalized allocation of therapy for our cardiovascular patients.

Inflammatory and Immune Mechanisms Participate in Atherothrombosis

Multiple strands of evidence, experimental and clinical, implicate inflammation in atherogenesis and its complications (1–8). Inflammatory status, monitored by C-reactive protein (CRP) concentrations measured with a high-sensitivity assay (hsCRP), can sharpen the prediction of first ever and recurrent cardiovascular events beyond that predicted by traditional risk algorithms (9,10). Targeting therapies with anti-inflammatory actions based on inflammatory status can reduce cardiovascular events (11). Reductions in events by statins may result in part from muting inflammation both by reduction in LDL and direct effects (12–14).

Despite these strong indications of the involvement of inflammation in atherosclerosis and the benefit of anti-inflammatory therapies, the use of agents such as the statins, which alter both the causal risk factor LDL and inflammatory pathways, cannot demonstrate

Corresponding Author: Peter Libby, MD, Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, Telephone: (617) 525-4383, Fax: (617) 525-4999, plibby@bwh.harvard.edu.

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unequivocally that a therapy that targets inflammation per se can reduce risk of atherosclerotic events. Moreover, high-dose statin treatment and other currently standard measures only prevent a fraction of recurrent events in survivors of myocardial infarction (MI) (15–17). This residual burden of events presents a pressing unmet medical need.

Cytokines Serve as Key Messengers of Inflammatory Signaling

Inflammation does not necessarily primarily trigger atherogenesis, but transduces the atherogenic effects of classical risk factors (8). Cytokines encompass a class of proteins that mediate inflammation and modulate immunity. One group of cytokines, the interleukins, bear that name because they were thought to mediate signaling between white blood cells (hence the name inter-leukin) (18). Cytokines contribute critically to atherosclerosis, among other inflammatory diseases (8,19,20).

Interleukin-1: A Primordial Pro-Inflammatory Cytokine

As indicated by its name, interleukin-1 (IL-1) figures among the first cytokines recognized. Several pathways converged on the identification of the molecules we now denote IL-1. The quest to identify the transferable sterile factor that induces fever (endogenous pyrogen activity) guided the purification of the protein we now know as IL-1 (21,22). The cloning of IL-1 cDNAs identified two related but functionally distinct isoforms: IL-1 α and IL-1 β (18) (Table 1). This pair of mediators has myriad effects in host defenses and in the pathogenesis of a wide variety of diseases. Notable effects of IL-1 on many cell types include the induction of prostaglandin production through the induction of cyclooxygenase-2 (COX-2); the elaboration of nitric oxide by elevation of levels of the inducible isoform of nitric oxide synthase (iNOS); induction of the expression of many cytokines, including augmenting its own gene transcription; the increased expression of leukocyte adhesion molecules and thrombogenic mediators; and the activation of cells involved in innate immunity, prominently including the mononuclear phagocytes (23) (Figure 1).

The Interleukin-1 Family and Its Regulation

As often in the case of pluripotent biological mediators, multiple levels of regulation control IL-1 action. The IL-1 family includes the structurally related IL-1 receptor antagonist (IL-1ra) (Table 1). (18) The balance between the pro-inflammatory IL-1 α and IL-1 β isoforms and this endogenous inhibitor (IL-1ra) represents one important level of control. The IL-1 α and IL-1 β isoforms have distinct functional profiles. IL-1 α typically resides on the cell surface and signals at short distances by direct contact. In contrast, IL-1 β can act at a distance.

Two receptors bind the principal members of the IL-1 family. IL-1 receptor I transduces IL-1 β signaling (Table 1). In contrast, the IL-1 receptor II (IL-1RII) binds the ligands but does not signal, as it lacks a cytoplasmic domain. IL-1RII thus functions as a “decoy,” providing yet another level of negative regulation of IL-1 signaling. IL-1 can induce its own gene expression in many cell types, including those implicated in atherogenesis (Figure 2A) (24,25). In the case of IL-1 α or IL-1 β inducing themselves or each other, a positive feedback

loop pertains (Figure 2B) (26). IL-1 α and IL-1 β can also increase the expression of IL-1ra, enabling negative feedback inhibition—a mechanism that prevents untrammelled IL-1 signaling.

The Inflammasome Activates Interleukin-1 β

In contrast to IL-1 α , the beta isoform requires processing to exert its biological actions. Several levels of control regulate the activation of IL-1 β . Pro-IL-1 β requires proteolytic processing to produce mature IL-1 β that possesses biological activity (Figure 3). The proteolytic enzyme responsible for producing the most mature, secreted IL-1 β , first called IL-1 β -converting enzyme, now bears the name caspase-1. This enzyme cleaves the 33 kD precursor of IL-1 to the active 17 kD form, analogous to processing of angiotensin I to the active angiotensin II by angiotensin-converting enzyme (Figure 3). Macrophages in human atherosclerotic plaques contain caspase-1 (Figure 2E) (27).

Caspase-1 in turn undergoes activation by a supramolecular assembly known as the inflammasome (Figure 3) (28,29). The inflammasome transduces inflammatory signals, culminating in cleavage of pro-caspase-1 to its active form, that in turn activates IL-1 β . Certain rare genetic diseases demonstrate the importance of the inflammasome in control of the overwhelming pro-inflammatory potential of the active cytokine. Muckle-Wells syndrome and cryopyrin-associated periodic syndrome (CAPS) arise from gain-of-function mutations in the inflammasome (30). These mutations markedly augment IL-1 β maturation and produce debilitating inflammatory diseases in children, indubitably due to overproduction of active IL-1 β .

The inflammasome generally requires two signals to assemble and act (28). The co-activators or triggers of the inflammasome include components of infectious agents. Crystals can also co-stimulate the inflammasome. For example, urate crystals implicated in the pathogenesis of gout also act via the inflammasome to enhance mature IL-1 β production (31). Cholesterol crystals also furnish a signal to the inflammasome (32,33). Human atheromata harbor activated inflammasomes (34). Other atherosclerosis-relevant stimuli that can augment inflammasome action include disturbed flow (such as prevails at sites of predilection to atheroma formation and complication) (35,36), and moderate hypoxia and acidosis (common in the core of plaques) (37,38).

Interleukin-1 Has Manifold Effects on the Cardiovascular System

A wealth of data implicates IL-1 in a number of cardiovascular diseases.(39,40) IL-1 induces inflammatory functions of human endothelial cells (ECs) (Figure 1).(41) IL-1 stimulates adhesion molecules that recruit leukocytes including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). IL-1 also induces chemokines such as MCP-1 (CCL-2), a chemoattractant for mononuclear phagocytes strongly implicated in inflammatory cardiovascular diseases. Cells in the atheroma produce IL-1 when exposed to inflammatory stimuli (Figure 2) (42,43). These observations led to the explicit hypothesis in the mid-1980s that IL-1 participates in atherogenesis (42).

IL-1 has multiple effects on human vascular smooth muscle cells (SMCs), a cell type intimately involved in atherogenesis (Figure 1). IL-1 can induce autocrine production of platelet-derived growth factor that can stimulate SMC proliferation.(44) IL-1 induces its own gene expression in many cell types, including ECs and SMCs, a powerful amplification loop denoted “autoinduction” (Figure 3) (24,25).

Treatment with IL-1 strongly induces SMCs and other cells to elaborate another cytokine, interleukin-6 (IL-6) (Figures 2C & 4).(45) IL-6 elicits the acute phase response: hepatocytes stimulated by IL-6 boost their synthesis of acute phase reactants, including fibrinogen and plasminogen activator inhibitor—molecules intimately involved in promoting thrombosis (fibrinogen) or limiting fibrinolysis (plasminogen activator inhibitor, PAI-1), as well as of CRP, a marker of inflammatory status (Figure 4) (46,47).

IL-1 therefore mediates an amplification loop whereby a single molecule of IL-1 can beget many molecules of IL-6, which in turn drive the overexpression of atherothrombosis mediators, such as those listed above (Figure 2C). Human genetic studies support causality of IL-6 in coronary heart disease (48,49). Thus, IL-1 resides upstream in the pathway that induces IL-6, a causal cytokine in atherothrombosis (Figure 4).

IL-1 alters functions of cardiac myocytes in addition to those of cells in the blood vessel wall. IL-1 impairs contractile function. This cytokine can aggravate ischemia-reperfusion injury and expansive cardiac remodeling after experimental MI (50,51). Experimental and small human studies suggest that antagonism of IL-1 can also benefit expansive remodeling after human MI, and limit the release of CRP following acute coronary syndromes (ACS) (52,53,54).

Numerous experiments involving genetically induced loss-of-function or gain-of-function of IL-1, manipulation of IL-1ra, and pharmacologic inhibition of IL-1 strongly implicate this cytokine in atherogenesis. Peri-adventitial application of IL-1 aggravates intimal thickening in pig arteries, implicating IL-1 in arterial hyperplasia, and IL-1 inhibition limits this response to injury (55,56). In hyperlipidemic mice, IL-1 generally promotes lesion formation, and interruption of IL-1 signaling limits atherogenesis (57–60). Deficiency of IL-1ra in mice augments arterial inflammation (61) and aneurysm formation (62), and hemizygous deficiency of IL-1ra limits early atherosclerosis and reduces macrophage content in hyperlipidemic mice.(63) IL-1 receptor 1–deficient hyperlipidemic mice have impaired expansive remodeling during lesion formation, attributable to reduced expression of the matrix metalloproteinase MMP-3 (stromelysin) (Figure 1) (64). Activated platelets can express IL-1 α and elaborate microparticles that bear functional IL-1 β , another link between this cytokine and atherothrombosis (65,66). The preponderance of data suggest a prominent contribution of IL-1 to the pathogenesis of multiple cardiovascular diseases (67).

Therapeutic Targeting of Interleukin-1 β

Given the wealth of findings that implicates inflammation in atherogenesis, and the residual burden of recurrent cardiovascular events in survivors of acute coronary syndromes—even when they receive contemporary standard medical care including high-dose statins, potent

anti-platelet combinations, and late generation stenting—the proposition that direct anti-inflammatory therapy could improve outcomes in such patients takes on considerable clinical urgency, beyond its theoretical interest. IL-1 β 's convincing links to pro-inflammatory diseases such as atherosclerosis highlighted this cytokine as a potential therapeutic target (68,69).

Several strategies can combat IL-1 action. Administration of the decoy receptor has not undergone substantive clinical development. The IL-1ra (anakinra) has conferred clinical benefit in some cases of rheumatoid arthritis, acute gouty arthritis, and diabetes mellitus. Anakinra requires daily injections, and blocks the actions of both IL-1 isoforms, potentially impairing host defenses and hence susceptibility to infection to a greater extent than selective isoform inhibition. In contrast to the IL-1ra, canakinumab, a human monoclonal antibody, selectively neutralizes IL-1 β but not IL-1 α , whereas IL-1ra blocks both. An anti-IL-1 α antibody has also entered clinical evaluation (70). Canakinumab benefits inflammatory diseases mediated by IL-1 β , including Muckle-Wells syndrome (71), rarer forms of juvenile inflammatory arthritis (72), and common diseases such as acute gouty arthritis (73). Canakinumab has a prolonged biological half-life that permits subcutaneous dosing every 3 months. Both anakinra and canakinumab appear well tolerated in most patients, although studies of the IL-1ra have not evaluated infection risk with long-term exposure of large populations. Moreover, as atherosclerosis-related stimuli activate the inflammasome that generates mature IL-1 β but not IL-1 α , the β isoform was chosen as the most disease-relevant target. In a phase II study of diabetic patients, canakinumab dose-dependently reduced plasma levels of fibrinogen, IL-6, and hsCRP.(74).

The Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS): Affirmation of the Role of Inflammation in Atherothrombosis

The strong biological basis presented above provided the rationale for a large-scale clinical trial that tested the hypothesis that administration of canakinumab to neutralize IL-1 β activity could improve outcomes in individuals who have sustained a prior MI.(75) CANTOS enrolled over 10,000 individuals at least one month post-acute MI in almost 40 countries. The protocol mandated aggressive use of all standard secondary prevention therapies for MI, including treatment with high-dose statins, aspirin and other anti-platelet agents, beta-adrenergic blocking agents, and agents that interrupt renin-angiotensin signaling, according to prevailing guidelines. The patients enrolled had residual inflammation despite these guideline-mandated secondary prevention measures, as indicated by levels of hsCRP above 2.0 mg/L. Stable post-MI patients eligible for CANTOS randomly received placebo or one of three doses of canakinumab, administered quarterly subcutaneously. The primary endpoint included non-fatal MI, non-fatal stroke, or cardiovascular death. The trial ended when approximately 1,400 events accrued in early 2017.

Canakinumab 150 mg subcutaneously every 3 months met CANTOS' primary endpoint with a 15 % reduction of non-fatal MI, non-fatal stroke, or cardiovascular death (76). The 150 mg and 300 mg doses had similar effects on this endpoint, yielding $p=0.007$ when combined. A

pre-specified expanded endpoint that included unstable angina requiring urgent revascularization showed a significant 17% reduction for the combined 150 mg and 300 mg groups. The 50 mg dose did not significantly lower events. The anti-IL-1 β treatment did not alter LDL, but the two higher doses lowered hsCRP by about 60%.

These results affirm the clinical importance and therapeutic relevance of the biological aspects of IL-1 β reviewed herein. Moreover, as the first large-scale blinded and placebo-controlled, randomized clinical trial that targeted inflammation but not lipids, CANTOS affirms the inflammatory hypothesis of atherosclerosis and sets the stage for a new era of cardiovascular therapeutics. Furthermore, the CANTOS results highlight the utility of assessing both LDL and hsCRP post ACS, as targeting either of these biomarkers can reduce recurrent events.

Indeed, we have entered an exciting era of “precision medicine,” which permits the allocation of therapies rationally based on readily measured biomarkers. Patients following ACS with LDL levels that remain above those desired despite statin therapy should receive consideration for adding a cholesterol absorption inhibitor (as validated in IMPROVE-IT (77)) or a PCSK9 inhibitor (as validated in FOURIER (78)). These patients represent *residual LDL risk*. Those who have persistently elevated inflammation despite a standard of care regimen, as gauged by an hsCRP greater than 2 mg/L, could receive canakinumab. These patients could be categorized as having *residual inflammatory risk* (79,80). Of note, FOURIER and CANTOS achieved a similar magnitude of event reduction on top of statins by targeting distinct aspects of residual risk.

Beyond CANTOS: Other Targets, Other Indications

IL-1 β represents only one of several potential avenues for targeting inflammation in atherosclerosis directly. As inflammatory signaling pathways have considerable redundancy, targeting any one pathway (such as IL-1 β signaling) may not block all inflammatory pathways implicated in atherogenesis. Following the success of CANTOS, a suite of studies should evaluate the role of other measures that manipulate inflammation and immunity in clinical scenarios beyond stable patients with prior myocardial infarction.

In this regard, some confusion has prevailed regarding interventions that target inflammation per se versus oxidative stress (81). The enzyme lipoprotein-associated phospholipase A₂ (LP-PLA₂) indeed arises primarily from inflammatory cells (e.g. macrophages) but acts to generate pro-oxidant lipid species. Two large clinical trials targeted LP-PLA₂ with a small molecule inhibitor (losmapimod) but failed to reduce cardiovascular events (82,83). Thus, these trials did not vitiate the inflammatory hypothesis as they focused primarily on reducing oxidative stimuli rather than inflammation itself. An inhibitor of p38 MAP kinase (losmapimod) likewise failed to reduce cardiovascular events in secondary prevention. Oxidative stress also activates p38 MAP kinase, and its inhibition does not interfere selectively with the IL-6 pathway causally implicated in cardiovascular events (84,85).

Clinical trials currently in progress target inflammation in ways that are less well defined. Colchicine, long used to mitigate certain inflammatory conditions, reduced cardiovascular

events in an unblinded, relatively small clinical trial (LoDoCo) (86). Two larger studies currently underway are following up this exciting and intriguing preliminary observation: the COLCOT and LoDoCo2 studies (87). The use of low-dose methotrexate has revolutionized the treatment of rheumatoid arthritis. The ongoing Cardiovascular Inflammation Reduction Trial (CIRT) evaluates whether this anti-inflammatory agent can improve cardiovascular outcomes in an at-risk population (88).

A wealth of experimental work over the last several decades has identified a number of other inflammatory targets with well-characterized mechanisms of action that merit consideration for future clinical investigation. Although IL-1 α has distinct cell biological and biochemical characteristics from the β isoform, it shares many pro-inflammatory actions including induction of IL-6. Antibody reagents that neutralize IL-1 alpha have entered clinical trials and hold considerable interest for cardiovascular indications (70,89). Tocilizumab, an antibody that selectively neutralizes IL-6, can treat several inflammatory diseases, but adversely perturbs the lipid profile. Other strategies for neutralizing IL-6 that lack such unwanted actions definitely merit evaluation for cardiovascular indications. A small trial in patients with acute coronary syndromes showed that tocilizumab reduced the excursion of CRP post primary coronary artery intervention (90).

Interleukin 18, a member of the IL-1 family, depends like IL-1 β on the inflammasome for activation. Several lines of evidence suggest that IL-18 participates in atherogenesis (91–93). This cytokine also deserves consideration for therapeutic targeting in atherothrombosis. As the inflammasome pathway activates the immature precursors of both IL-1 β and IL-18, inhibition of the NLRP3 inflammasome could offer an approach to simultaneous limitation of the action of this pair of pro-inflammatory cytokines.(94–96) Yet, given the potential risks of interfering with host defenses, such dual effects might have more liability than inhibition of a single isoform of a single cytokine.

Considerable data implicate CD40 and its ligand CD154 in cardiovascular and metabolic diseases (97–101). While monoclonal antibody strategies encountered thrombotic complications, a late-generation antisense oligonucleotide approach has promise as an approach to limiting the action of this signaling dyad (102). A small molecule inhibitor of CD40 interaction with its signal transducing partner TRAF also holds interest in this regard (103).

Many studies have implicated tumor necrosis factor (TNF) in inflammatory diseases including atherosclerosis (104,105). Like methotrexate, anti-TNF strategies have proven highly successful in certain rheumatologic and inflammatory diseases. Yet, anti-TNF strategies can adversely affect lipid profiles. Moreover, despite preclinical promise, trials that targeted TNF in patients with heart failure showed no benefit, rather a possible hazard (105). These considerations have limited interest in targeting TNF in cardiovascular patients.

Experimental evidence implicated the monocyte chemoattractant MCP-1 (CCL2) and its receptor CCR2 in atherogenesis (106–108). Currently, research regarding monocyte heterogeneity in cardiovascular diseases has flourished (109–112). In particular, a pro-inflammatory subset of monocytes recruited via the chemokine receptor CCR2 has proven

functionally important in a number of cardiovascular conditions. As monoclonal antibodies that can neutralize CCR2 have entered clinical use, they also merit clinical evaluation to quell cardiovascular inflammation.

Any intervention that interferes with innate immunity, such as those delineated above, might increase susceptibility to infections and/or impair tumor surveillance. Therefore, the clinical applications of anti-inflammatory therapies require careful consideration of potential adverse effects. Indeed, CANTOS revealed a small but significant increase in fatal infections (an excess of about 1 per 1000 patient-years) (76). Analysis of infection liability in the over 6,700 patients exposed to canakinumab will inform regarding risk factors for infection and help to mitigate this risk. The decades of experience with anti-inflammatory interventions in rheumatologic and other diseases provide guidance in this regard, as we move towards clinical application in the cardiovascular arena of biological and other strategies to modulate the inflammatory response.

One might have expected an increase in cancer due to impaired tumor surveillance due to treatment with canakinumab, as anti-TNF strategies do entail a risk for lymphoma. Yet, in CANTOS actually showed a substantial and canakinumab-dose-dependent reduction in fatal malignancy ($p=0.0007$) (113). Canakinumab (300 mg/3 months) produced a 67% fall in incident lung cancer, and a 77% decrease in fatal lung cancer ($p=0.0002$.) The cancer benefit in this trial unlikely represents an effect on cancer initiation, although IL-1 may contribute to oncogenesis in some circumstances (114). Rather, IL-1 blockade may impede the invasion and metastasis of existing cancers. IL-1 can induce a key proteinase involved in cancer spread, matrix metalloproteinase-2 (MMP-2), implicated in breaching the basement membrane by cancers (Central Illustration) (115). IL-1 can also promote epithelial-to-mesenchymal transition implicated in tumorigenesis (116). Most cancer treatments entail cardiovascular risk. Canakinumab appears capable of reducing risk of cancer fatalities in the inflamed population studied while producing cardiovascular benefit rather than hazard.

Beyond the chronic phase of atherosclerosis in secondary prevention, inflammation participates pivotally in other clinical scenarios. Cytokines modulate the healing of acute ischemic myocardial injury experimentally (53). Therefore, some of the anti-cytokine strategies summarized above might foster beneficial healing after an ischemic insult in patients with ACS. For example, treatment with an anti-IL-1 beta antibody limits mobilization of pro-inflammatory monocytes to the infarcted myocardium and improves left ventricular function following coronary artery ligation in mice (117). The data available from the over 1,400 primary endpoint events in CANTOS, many of them ACS, will provide safety data to inform the use of canakinumab administration during ACS, a highly inflamed patient population excluded from CANTOS which enrolled only individuals at least 30 days following an ACS. Interventions that target CCR2 also promote healthier healing of the ischemic myocardium (118).

Conclusion - Entering an Era of Targeting Inflammation in Cardiovascular Diseases

In conclusion, we have entered an exciting era in which we can reap in the clinic the benefits of decades of research into the participation of immune and inflammatory pathways in cardiovascular disease (8). While extrapolation of preclinical studies to humans requires considerable caution (119), the burden of unmet need and residual risk in our patients mandates careful consideration of novel approaches to targeting inflammatory pathways.

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Abbreviations

hsCRP	high-sensitivity C-reactive protein
IL	interleukin-1
MI	myocardial infarction
ACS	acute coronary syndromes –
cDNA	complementary DNA
ICAM-1	intercellular adhesion molecule-1
VCAM-1	vascular cell adhesion molecule-1
SMC	smooth muscle cells
EC	endothelial cells
MMP	matrix metalloproteinase

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Condensed Abstract

Inflammatory pathways drive atherogenesis and link traditional risk factors to atherosclerosis and its complications. Interleukin-1 beta (IL-1 β) has emerged as an actionable mediator in prevention of recurrent cardiovascular events. Intrinsic vascular wall cells and lesional leukocytes alike can produce this pro-inflammatory cytokine. Local stimuli in the plaque boost the generation of active IL-1 β through the action of a molecular assembly known as the inflammasome. Therapies that interfere with IL-1 action can improve cardiovascular outcomes, ushering in a new era of anti-inflammatory therapies for atherosclerosis. Biomarker-directed application of anti-inflammatory interventions promise to personalize allocation of therapy for our cardiovascular patients.

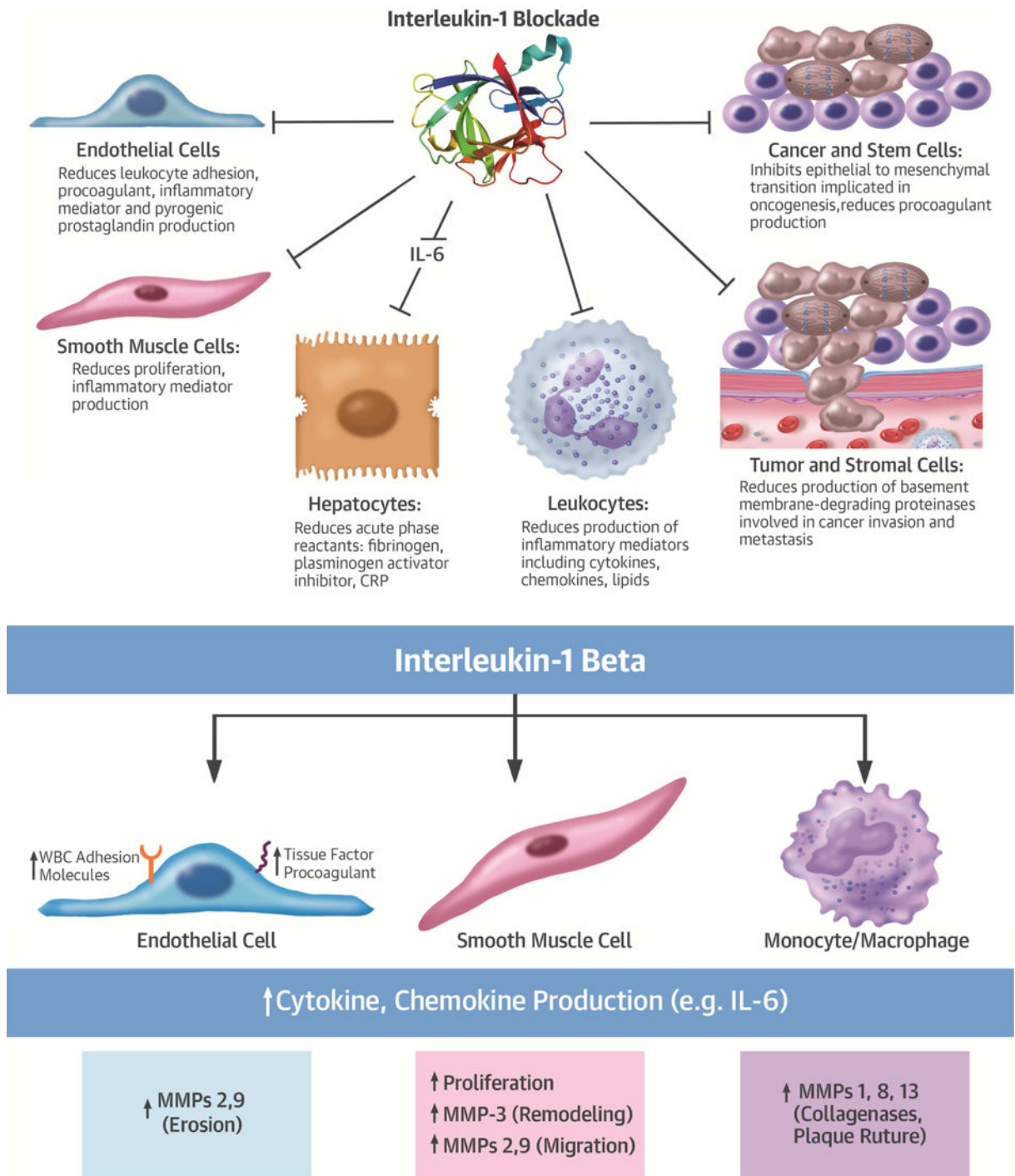


Figure 1. Selected actions of IL-1 related to atherosclerosis

IL-1 β acts on many cell types and organs, including those involved in atherogenesis such as vascular endothelial and smooth muscle cells, and macrophages. IL-1 β elicits the functions shown, among many others. Blockade of IL-1 induced MMP-2 (Type IV collagenase) may contribute to the marked drop in cancer incidence and mortality seen in CANTOS by

impeding tumor invasion through the collagen IV-rich basement membrane and thus limiting metastasis. MMP: matrix metalloproteinase, WBC: White blood cell.

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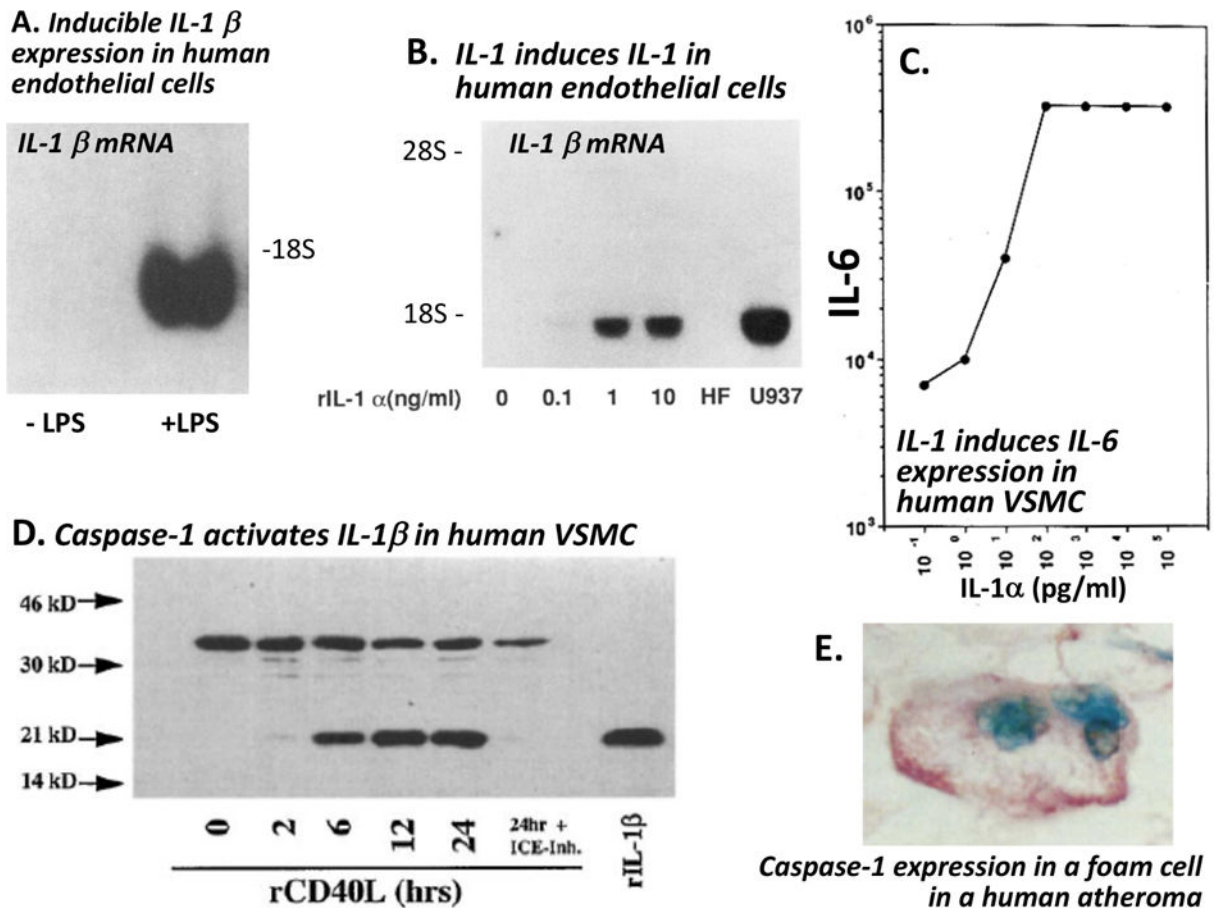


Figure 2. IL-1 activates human vascular wall cells. A: Human endothelial cells express IL-1 β after exposure to bacterial endotoxin

Cultured human endothelial cells were exposed to *E. coli* endotoxin (LPS) for 24 hours. IL-1 β mRNA was evaluated by Northern blotting (42).

B: IL-1 induces IL-1 in human vascular cells. Cultured human endothelial cells were exposed to recombinant IL-1 α at the concentrations noted for six hours. IL-1 β messenger RNA was visualized by Northern blotting.(25) Such auto induction of interleukin one isoforms occurs in human vascular smooth muscle cells and in macrophages as well (24,120). Either IL-1 isoform can induce the other or itself.

C. IL-1 induces IL-6 expression in human vascular smooth muscle cells (VSMC.) Human the SMC were exposed to IL-1 α at the concentration shown for 24 hours. IL-6 activity released by the cells is reported in arbitrary units on a logarithmic scale. IL-1 also induces IL-6 in human endothelial cells.(121).

D. Caspase-1 activates IL-1 β in human VSMC stimulated with recombinant CD40 ligand (rCD40L.) The 33 kD pro-IL-1 β concentration declines as the active 17 kD mature form accumulates. The conversion depends on caspase-1 as shown by treatment with the caspase-1/IL-1 β converting enzyme (ICE) inhibitor ZVAD (122).

E. Foam cells in a human atheroma expresses caspase-1. This representative view of typical human atherosclerotic plaque shows immunohistochemical staining for caspase-1, the IL-1 β converting enzyme (pink reaction product).(27) (Republished with permission from the following sources: 1) Libby P, Ordovàs JM, Auger KR, Robbins H, Birinyi LK, Dinarello CA. Endotoxin and tumor necrosis factor

induce interleukin-1 gene expression in adult human vascular endothelial cells. *Am J Pathol* 1986;124:179–186. 2) Geng Y-J, Libby P. Evidence for apoptosis in advanced human atheroma. Co-localization with interleukin-1 beta-converting enzyme. *Am J Pathol* 1995;147:251–266. 3) Loppnow H, Libby P. Adult human vascular endothelial cells express the IL6 gene differentially in response to LPS or IL1. *Cell Immunol* 1989;122:493–503. 4) Warner SJC, Auger KR, Libby P. Interleukin-1 induces interleukin-1. II. Recombinant human interleukin-1 induces interleukin-1 production by adult human vascular endothelial cells. *J Immunol* 1987;139:1911–1917. Copyright © [1987] The American Association of Immunologists, Inc. 5) Dinarello CA, Ikejima T, Warner SJC et al. Interleukin-1 induces interleukin-1. I. Induction of circulating interleukin-1 in rabbits in vivo and in human mononuclear cells in vitro. *J Immunol* 1987;139:1902–1910. Copyright © [1987] The American Association of Immunologists, Inc. 6) Warner SJC, Auger KR, Libby P. Human interleukin 1 induces interleukin 1 gene expression in human vascular smooth muscle cells. *J Exp Med* 1987;165:1316–1331. 7) Schoenbeck U, Mach F, Bonnefoy JY, Loppnow H, Flad HD, Libby P. Ligation of CD40 activates interleukin 1beta-converting enzyme (caspase-1) activity in vascular smooth muscle and endothelial cells and promotes elaboration of active interleukin 1beta. *Journal of Biological Chemistry* 1997;272:19569–19574. © the American Society for Biochemistry and Molecular Biology.)

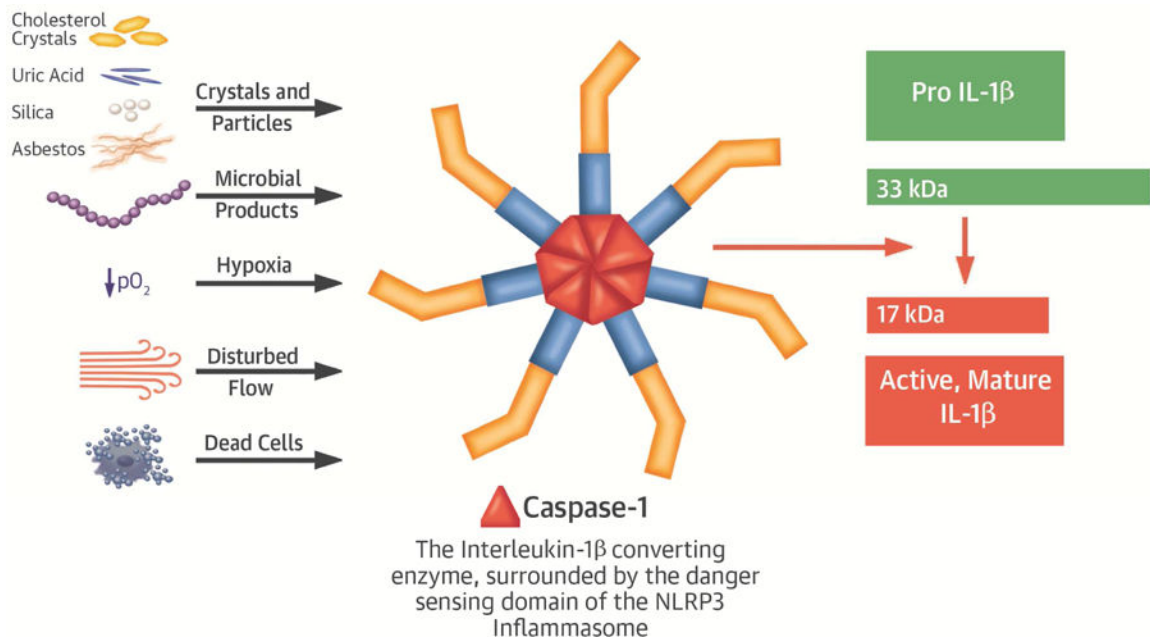


Figure 3. The inflammasome senses danger signals and activates IL-1 beta by proteolytic processing to the mature form by caspase 1

Transcription of the interleukin-1 (IL-1) beta (β) gene produces pro-IL-1 β , a 33 kiloDalton (kD) protein that lacks biological activity. This precursor undergoes cleavage by an enzyme known as IL-1 β converting enzyme (ICE) or caspase-1, to produce the active form of the cytokine with a molecular weight of 17 kD. The activity of caspase 1 depends on the NLRP3 inflammasome that senses danger signals such as those shown.

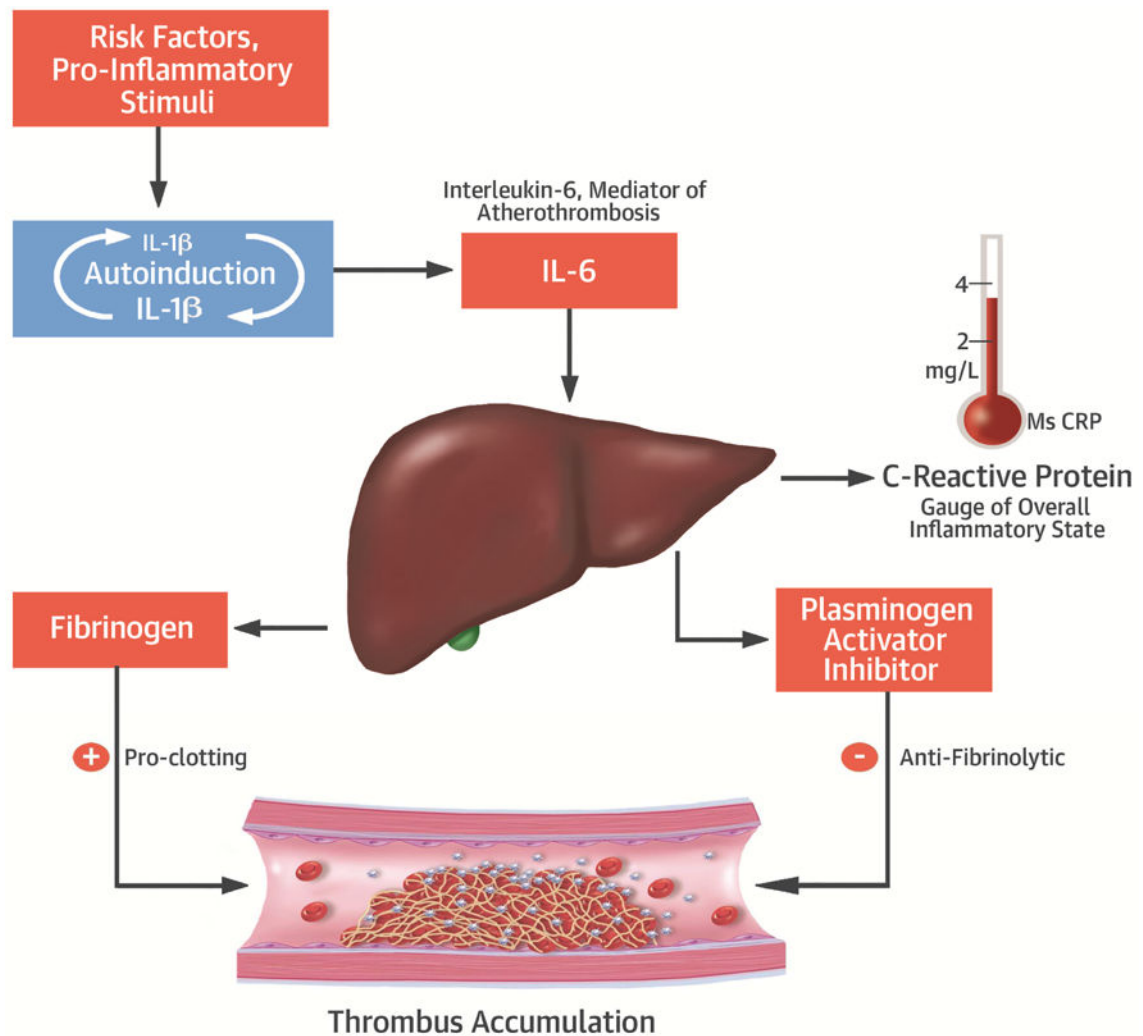
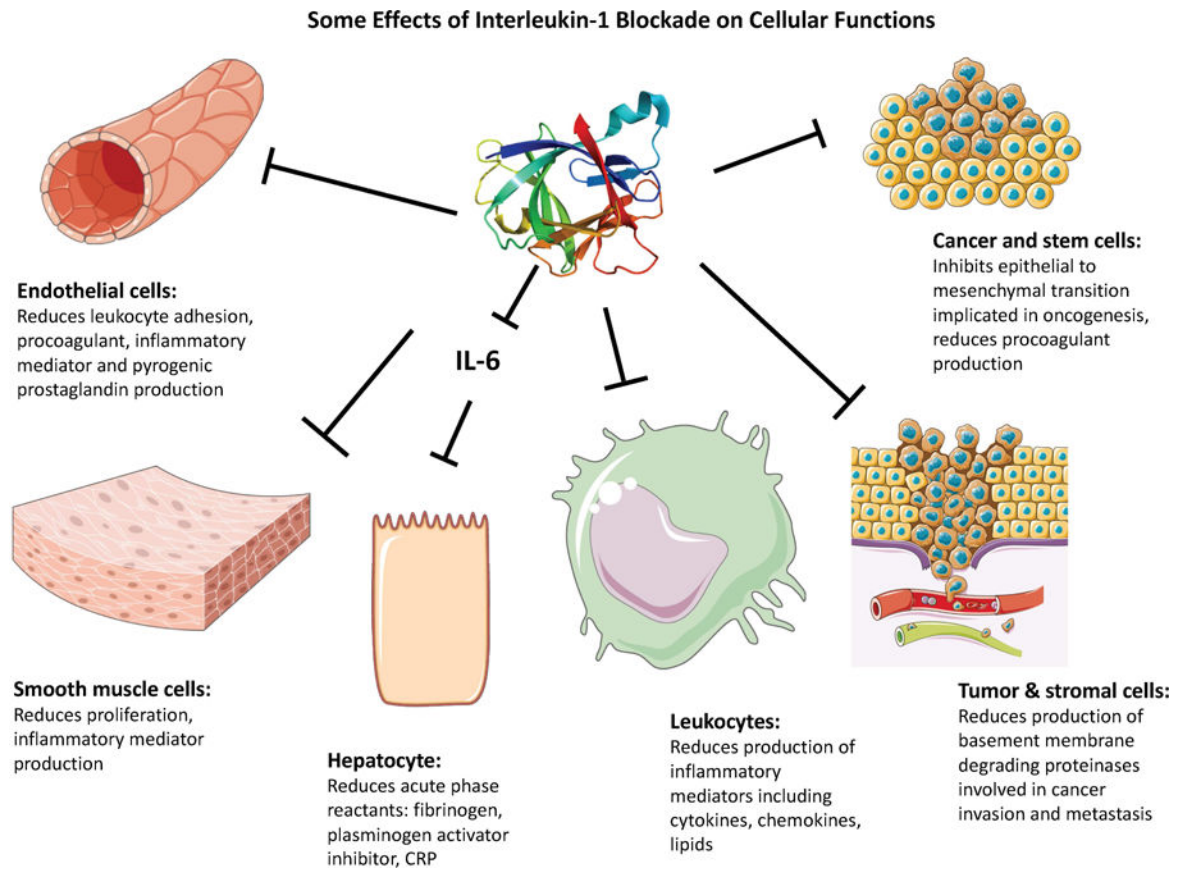


Figure 4. Amplification cascades boost IL-1 actions implicated in atherothrombosis
 Interleukin-1 (IL-1) can induce its own gene expression in many cell types, including those implicated in atherosclerosis. This property provides a positive feedback amplification loop for boosting IL-1 levels at sites of inflammation. IL-1 also strongly augments the production of IL-6 by various cell types. IL-6 mediates the acute phase response through which the liver produces proteins that participate in host defenses, but that also augments thrombosis and inhibits fibrinolysis as explained in the text.



Central Illustration. Some Effects of Interleukin-1 Blockade on Cellular Functions

Interleukin-1 exerts many actions on cells that can contribute to pathogenesis diseases including atherosclerosis, thrombosis, oncogenesis, and invasion and metastasis of tumors. Many of the effects of interleukin-1 on hepatocytes, including the induction of the acute phase response, depend on the intermediary of interleukin 6 (IL-6), a cytokine potently induced by interleukin 1. Strong human genetic evidence implicates IL-6 as a causal factor for atherothrombosis.

Table 1

The IL-1 and receptor family members

Cytokine:	Comments:
Interleukin-1 alpha (IL-1α)	IL-1 α generally remains associated with the cell surface or is released by dying cells and usually acts at short distances by juxtacrine or paracrine signaling
Interleukin-1 beta (IL-1β)	IL-1 β acts primarily extracellularly as a soluble mediator
Interleukin-1 receptor antagonist (IL-1ra)	IL-1ra blocks the signaling receptor that binds either IL-1 α or β
Receptors:	
Interleukin-1 receptor 1 (IL-1r1)	IL-1 receptor 1 binds both isoforms
Interleukin-1 receptor 2 (IL-1r2)	IL-1 receptor 2 does not signal and thus acts as an inhibitory decoy

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Table 2Selected targets for limiting cardiovascular inflammation beyond IL-1 β

Inflammasomes
IL-1 α
IL-6
IL-18
IL-33
TNF
CD40/CD40L (CD164)
TRAF-6
Lipid mediators of resolution

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