

JACC REVIEW TOPIC OF THE WEEK

Inflammation, Immunity, and Infection in Atherothrombosis



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ABSTRACT

Observations on human and experimental atherosclerosis, biomarker studies, and now a large-scale clinical trial support the operation of immune and inflammatory pathways in this disease. The factors that incite innate and adaptive immune responses implicated in atherogenesis and in lesion complication include traditional risk factors such as protein and lipid components of native and modified low-density lipoprotein, angiotensin II, smoking, visceral adipose tissue, and dys-metabolism. Infectious processes and products of the endogenous microbiome might also modulate atherosclerosis and its complications either directly, or indirectly by eliciting local and systemic responses that potentiate disease expression. Trials with antibiotics have not reduced recurrent cardiovascular events, nor have vaccination strategies yet achieved clinical translation. However, anti-inflammatory interventions such as anticytokine therapy and colchicine have begun to show efficacy in this regard. Thus, inflammatory and immune mechanisms can link traditional and emerging risk factors to atherosclerosis, and offer novel avenues for therapeutic intervention. (J Am Coll Cardiol 2018;72:2071-81)

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Considerable evidence supports the role of inflammation and immunity in atherosclerosis (1,2). The authors include panelists in a series of workshops convened in cooperation with the National Heart, Lung, and Blood Institute to consider and weigh the evidence with regard to these topics. This discussion distills the proceedings of these multiple interactions.

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Manuscript received June 25, 2018; revised manuscript received August 1, 2018, accepted August 6, 2018.



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**ABBREVIATIONS
AND ACRONYMS****hsCRP** = high-sensitivity
C-reactive protein**IL** = interleukin**LDL** = low-density lipoprotein**PAMP** = pathogen-associated
molecular pattern**Th** = helper T-cell subtype**TLR** = toll-like receptor

Convergent streams of convincing data from animal experiments, observations on human atheromata, and clinical biomarker studies all support the importance of immune and inflammatory pathways in the pathogenesis of this disease. During much of the 20th century, research focused on cholesterol and subsequently lipoproteins as the key mechanism in establishing fatty lesions in arteries (3). The advent of a cell biological approach added to the biochemical discoveries related to cholesterol in the 1970s (4-6). Although low-density lipoprotein (LDL) certainly contributes causally to atherothrombosis, other lipoprotein fractions, non-lipid-related genetic variants, and lifestyle also influence this disease (7,8). Indeed, almost one-half of a population can harbor subclinical atherosclerosis without a high burden of traditional risk factors (9). Thus, elevated LDL levels alone cannot account for the entire burden of atherosclerosis.

The concept of atherosclerosis as a proliferative disorder of arterial smooth muscle cells gained prominence as a pathogenic pathway independent of cholesterol (10). Schemata of the pathogenesis of atherosclerosis posited a denuding injury to the endothelium followed by the deposition of platelets and release of the protein platelet-derived growth factor that would stimulate the migration and proliferation of smooth muscle cells (5,6). Extracellular matrix elaborated by these cells would entrap plasma-derived lipids, giving rise to the atheroma. Some viewed atherosclerosis as akin to leiomyomata, a benign tumor of smooth muscle cells arising by a monoclonal or monotypic pathway (11-13). Indeed, initial iterations of the “response to injury” hypothesis depicted atherosclerosis as a bland process, devoid of inflammation (5,6,14).

The advent of monoclonal antibody technology in the 1980s permitted more rigorous identification of the cell types that accumulate in human atherosclerotic plaque (15,16). These studies identified the foam cells in the atherosclerotic plaque as arising primarily from mononuclear phagocytes (Figure 1). Ironically, recent data suggest that smooth muscle cells may indeed give rise to foam cells through metaplasia, with cellular and molecular characteristics in common with mononuclear phagocytes (17). This concept unites the proliferative concept of atherogenesis with inflammatory pathways.

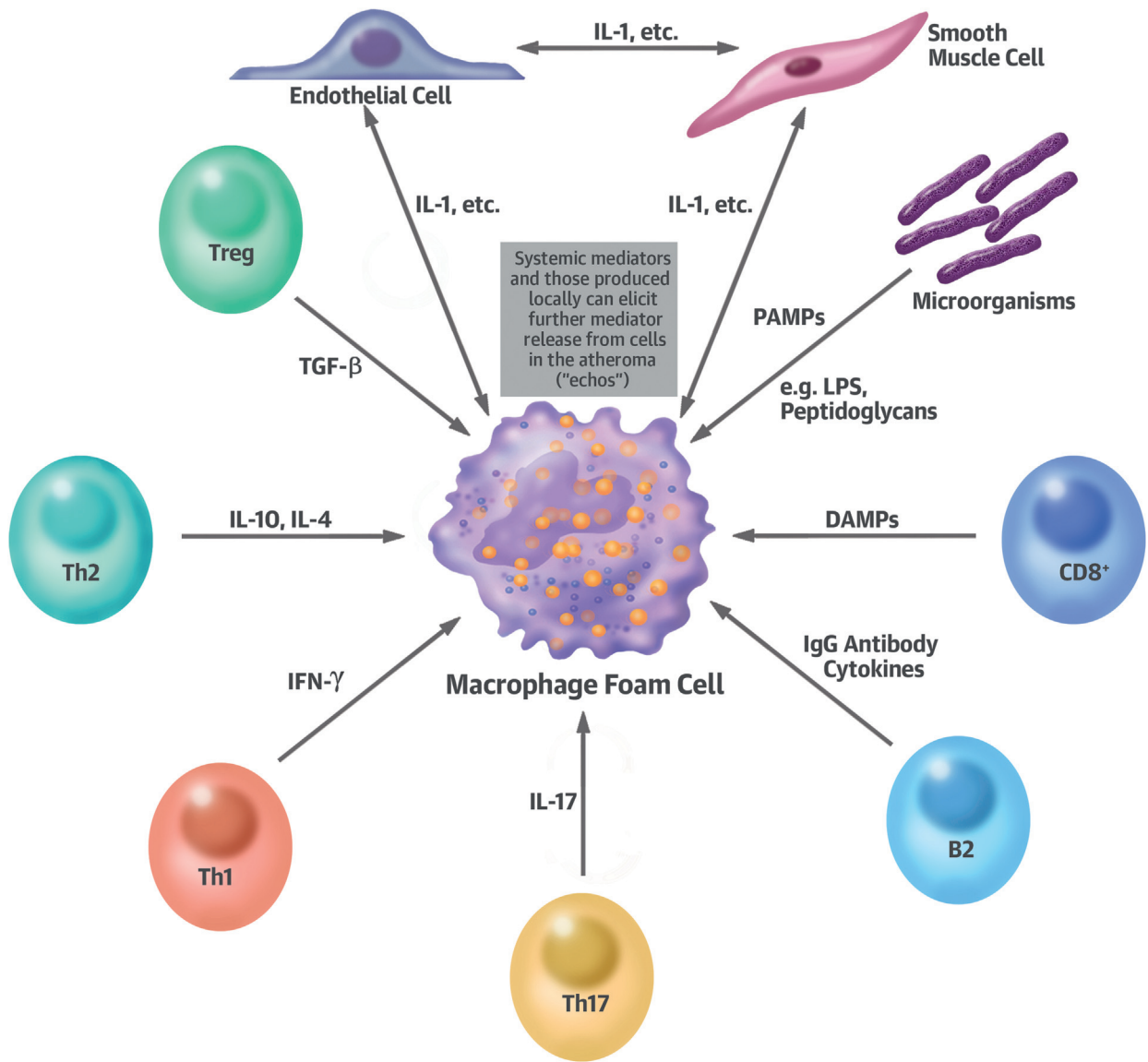
Soon after the unambiguous demonstration of macrophage markers on many foam cells in atheromata, Hansson’s group and others described the presence of a smaller population of T lymphocytes

within the plaque (16,18). Although the plaque T-cell population is scant in number, the expression of class II histocompatibility antigens on neighboring smooth muscle cells provided evidence for the functional activity of these lymphocytes in the arterial plaque (19,20). The induction of these molecules, critical in the afferent limb of the cellular immune response, depends in large measure on interferon gamma, a signature cytokine of helper T cells of the Th1 subtype (21). Far from being mere “fellow travelers,” the T cells in plaque thus appear to function immunologically. Such morphological observations in human plaque opened the door to a plethora of experimental studies which showed that adaptive immune responses modulate atherogenesis (22-25). These and other studies established that cells of the immune system, both the innate (macrophages) and adaptive (T cell and other lymphocytes) limbs, contribute to atherosclerosis (Figure 1).

Simultaneously with the identification of cells of the innate and adaptive immune systems in the atherosclerotic plaque, research progressed on the signals exchanged among the leukocyte invaders into the artery wall and the intrinsic vascular wall cells: smooth muscle and endothelium. Such interactions connect the immune cells to altered arterial biology. Cytokines (protein mediators of inflammation) mediate the exchange of signals between leukocytes and intrinsic arterial wall cells (26,27). The interleukins (ILs), so-called because they were originally believed to mediate cross-talk between leukocytes, can also arise from vascular smooth muscle and endothelial cells (28,29). The leukocytes and vascular cells in the plaque constitute the protagonists in inflammation during atherogenesis, and the cytokines provide the dialogue by which these actors communicate. A subset of cytokines, the chemokines, mediates migration of cells in the plaque (30,31). Chemokines participate in recruitment of the leukocytes and their directed migration from the luminal surface into the plaque; they can also stimulate smooth muscle cell migration.

We now recognize that the inflammatory and immune response can operate positively, to promote disease, or negatively, to modulate deleterious action and promote resolution or repair of lesions (Figure 1) (32). In addition to pro-inflammatory subsets of mononuclear phagocytes, reparative or less inflammatory populations also participate in the modulation of atherogenesis (33). Single-cell analyses indicate a much greater complexity of leukocyte subtypes than previously recognized (34-36). T cells not only incite inflammatory responses but can inhibit them (e.g., regulatory T cells, Th2 lymphocytes). B2 lymphocytes

FIGURE 1 Infection and Immunity in Atherogenesis



This figure depicts a trio of key cell types of the atheroma: endothelium, smooth muscle, and macrophage foam cells representative of the leukocytes found in plaques. These cells found within the atherosclerotic plaque can produce cytokines, notably interleukin (IL)-1, which can contribute to a positive feedback loop because IL-1 can induce its own gene expression in the source cell, an autocrine pathway, or activate neighboring cells through juxtacrine or paracrine pathways. Systemic mediators or these locally produced cytokines can elicit further mediator release from cells in the atheroma producing local echoes of systemic inflammation. The surrounding cells represent the lymphocytes that interact with cells within the atheroma and some main mediators by which they can modulate the local inflammatory and immune response within the plaque. Regulatory T cells (Treg) elaborate transforming growth factor beta (TGF-β), a mediator that exerts anti-inflammatory and pro-fibrotic actions on many cell types. The T-cell helper (Th) 2 lymphocytes can mute inflammatory responses and promote resolution or healing responses through the elaboration of IL-10 and IL-4. The Th1 lymphocytes can release interferon gamma (IFN-γ) that can potentially produce pro-atherogenic functions of the 3 major cell types depicted in the arterial plaque. The induction of class II major histocompatibility molecules on the surface of antigen-presenting cells in the plaque such as the macrophage can, in turn, enhance their ability to stimulate the afferent limb of the adaptive immune response. Th17 cells elaborate IL-17, which has mixed effects on atheromata, perhaps promoting inflammation but also augmenting fibrosis. B lymphocytes can also modulate atherosclerosis. B1 cells (not depicted here) can secrete natural immunoglobulin M antibodies that appear atheroprotective. Many such natural antibodies recognize epitopes associated with modified lipoproteins. B2 cells can elaborate cytokines and immunoglobulin G (IgG) antibodies that can stimulate atherogenesis, based on observations in mice. CD8 lymphocytes can kill virally infected cells, including those in the atheroma releasing damage-associated molecular patterns (DAMPs) that can augment inflammatory activation of many cell types through engagement of the toll-like receptors. Microorganisms themselves, as depicted by the bacillus in the diagram, can provide pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) or peptidoglycans that activate cells in the atherosclerotic plaque through engagement of the toll-like receptors. This complex network involves interactions between innate immune pathways and both humoral and cellular adaptive immunity.

tend to aggravate atherogenesis, whereas products of B1 lymphocytes exert antiatherogenic effects (37). A recognized subclass of B lymphocytes known as innate response activator B cells can aggravate atherogenesis in mice by augmenting Th1 responses, illustrating the links between cells that participate in humoral and T-cell-mediated immunity (38). Anti-inflammatory cytokines (e.g., IL-10, IL-4) tend to mitigate the pro-inflammatory actions of cytokines such as IL-1 and interferon gamma. Beyond anti-inflammatory adaptive and innate responses, we now recognize that lipid mediators of resolution can also regulate atherogenesis (39,40).

Accumulating evidence supports roles for myeloid cells in atherothrombosis (41). Mutations can occur in bone marrow stem cells with age that give rise to clones of leukocytes in peripheral blood. As expected, those who have these clones of mutant white blood cells have increased susceptibility to development of hematologic malignancies. Unexpectedly, people who have such leukocyte clones have a markedly increased incidence of cardiovascular events. This entity, known as clonal hematopoiesis of indeterminate potential (as few who have these clones will actually develop leukemia), constitutes a potent newly recognized risk factor for cardiovascular disease. These recent findings highlight another link between innate immune cells and atherothrombosis (42-44).

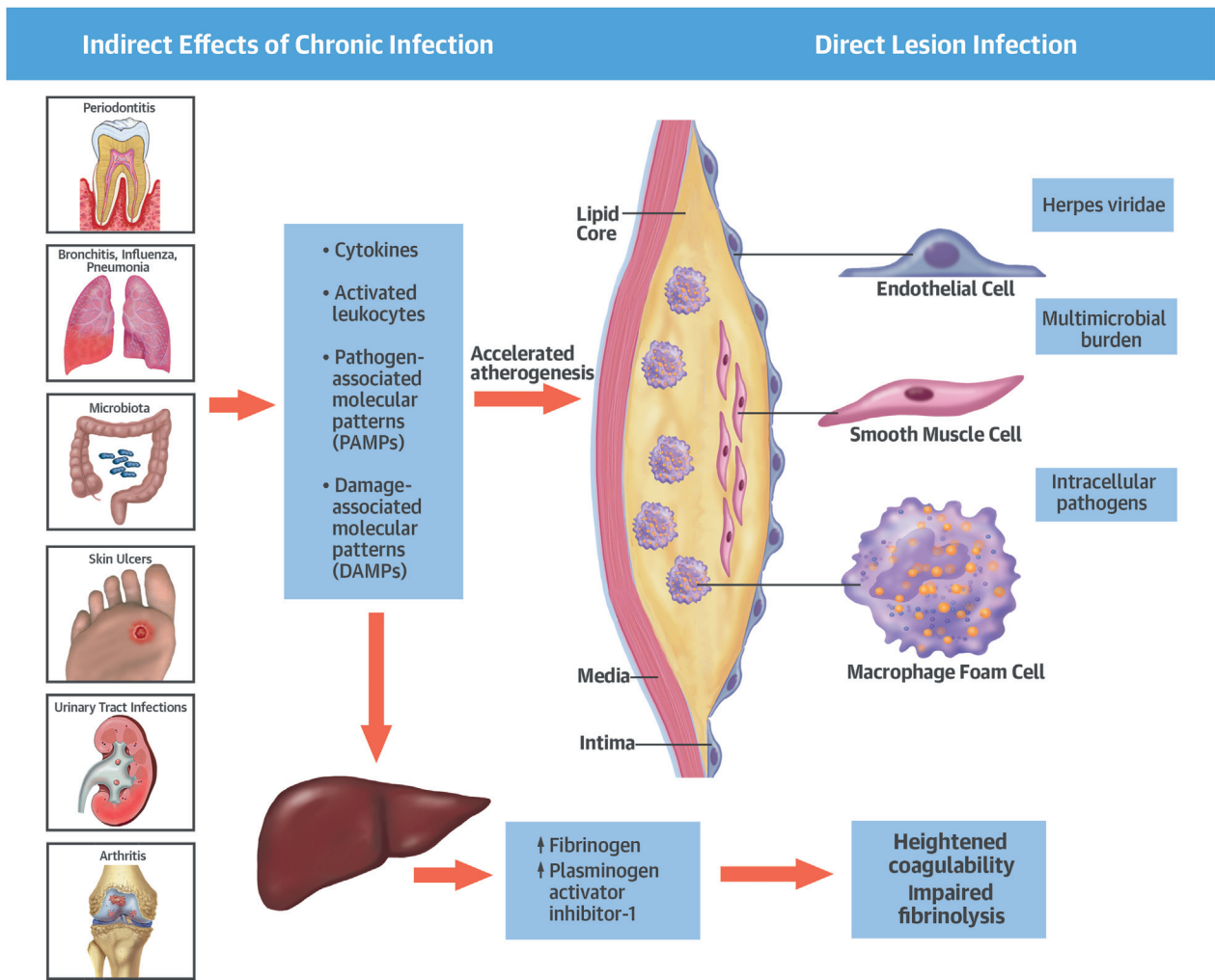
In sum, overwhelming evidence supports a complex, multilateral, and tightly regulated series of immune and inflammatory responses that initiate atherogenesis and engage in a “tug of war” during the phase of progression of atherosclerosis with competing positive and negative influences contributing to the prolonged incubation period of this disease. Ultimately, these pathways can regulate aspects of the plaque that lead to their disruption, providing a nidus for thrombosis (45,46). Inflammatory and immune mediators also influence coagulation and fibrinolysis, providing another dimension to their ability to modulate the complications of atherosclerosis (47). Rather than replacing or challenging traditional risk factors, the pathways of inflammation and immunity provide mechanistic explanations that connect these risk factors to altered behavior of vascular wall cells that give rise to the disease and its complications. Thus, the notion that inflammation, immunity, and infection can contribute to atherogenesis or trigger atherosclerotic events in no way challenges the concept that cholesterol contributes critically to disease development; rather, it adds another dimension to an extraordinarily complex pathobiological process.

WHAT TRIGGERS INFLAMMATION AND IMMUNITY IN ATHEROTHROMBOSIS?

Elucidation of immune and inflammatory pathways that function during atherogenesis does not illuminate the triggers that unleash their function. LDL can activate T cells, providing one link between a well-established traditional risk factor and adaptive immunity (48). Although a very large amount of literature supports the oxidation of LDL and its pro-inflammatory actions, antioxidant therapies have consistently failed to alter outcomes in humans with atherosclerosis. Thus, to date, the concept of LDL oxidation has not proven actionable in the clinic. Angiotensin II can act as a pro-inflammatory stimulus (49). Adaptive immunity also participates in experimental hypertension, linking high blood pressure with immune and inflammatory pathways (50). Visceral adipose tissue provides another potential stimulus to plaque inflammation through the elaboration of pro-inflammatory cytokines (51-53). These observations link obesity and insulin resistance to inflammatory pathways in arterial disease (54). Such examples illustrate how traditional risk factors can interface with the immune and inflammatory systems in ways that modulate atherogenesis.

The immune and inflammatory responses likely evolved primarily to protect the organism from infectious agents. Seeking triggers of these host defense mechanisms, investigators have expended considerable effort through the decades investigating the possible role of infectious agents in atherosclerosis and its complications (55-58). These studies identified markers of nucleic acid and antigens of viral and bacterial pathogens within atherosclerotic plaques. Moreover, bacterial products can stimulate vascular inflammation (59,60). In the extreme example, Gram-negative bacterial endotoxins strongly elicit inflammatory responses from endothelial cells, which explains much of the pathogenesis of septic shock (Figure 2) (28). Lower levels of endotoxemia indeed associate with cardiovascular risk (61-63). Seroepidemiological studies provided evidence that linked infections (notably with *Chlamydia pneumoniae*) with susceptibility to cardiovascular disease and atherosclerotic complications (57,64,65). However, prospective and better-controlled studies did not support many of the initial observational, seroepidemiological studies that linked infection to atherosclerosis (66,67). A wave of clinical trials, including rigorous and sufficiently powered investigations, examined whether antibiotic treatment could prevent cardiovascular

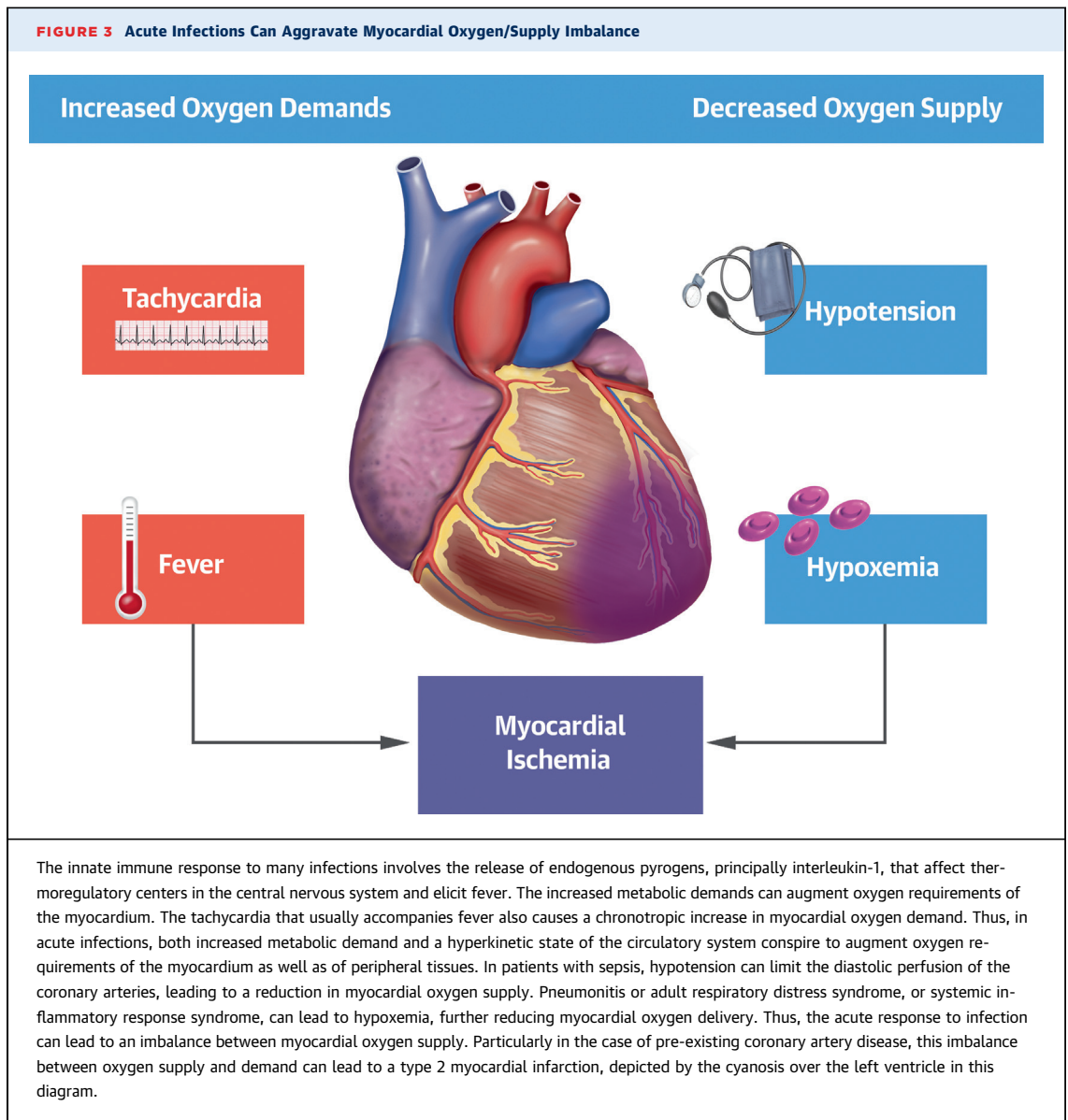
FIGURE 2 Chronic Infections Can Accelerate Atherogenesis and Set the Stage for Thrombotic Complications Directly and Indirectly



The **left side** of this diagram lists a number of examples of chronic infections or collections of microorganisms that can produce indirect effects on the cardiovascular system. For example, chronic infections can release cytokines, activate leukocytes, and elaborate pathogen-associated molecular patterns such as lipopolysaccharides or peptidoglycan components of bacterial cell walls. Tissue injury can produce and provoke the release of damage-associated molecular patterns. Cytokines, through their cognate receptors, and pathogen-associated and damage-associated molecular patterns, often through toll-like receptors, can contribute to the activation of cells involved in atherogenesis, including the intrinsic inhabitants of the normal artery wall, the endothelial and smooth muscle cells, or the leukocytes that accumulate in atherosclerotic lesions such as the macrophage foam cell. Activated white blood cells can also elaborate inflammatory mediators, including cytokines, lipid mediators, and reactive oxygen species that may enhance atherogenic functions of cells within the atheroma. Cytokines through the intermediary of interleukin-6 can elicit the acute-phase response from hepatocytes. Among the acute-phase reactants elaborated by hepatocytes during this response, fibrinogen can increase coagulability, and plasminogen activator inhibitor-1 can impair fibrinolysis. These changes in the fluid phase of blood can hasten the formation of thrombi and impair their resolution by inhibiting fibrinolysis. The **right side** of the diagram indicates various viral and microbial pathogens that studies have implicated in atherogenesis. The infection of endothelial and smooth muscle cell and of lesion-associated leukocytes can directly promote inflammation within the plaque.

events in patients with established atherosclerosis. The most rigorous and best-powered of these studies found no reduction in cardiovascular events with treatment with macrolides, such as azithromycin, or fluoroquinolones, such as gatifloxacin, in the

patients tested (68,69). Because macrolide use may associate with increased sudden death or ventricular tachyarrhythmias, their unwarranted use for cardiovascular prevention could cause hazards (70).

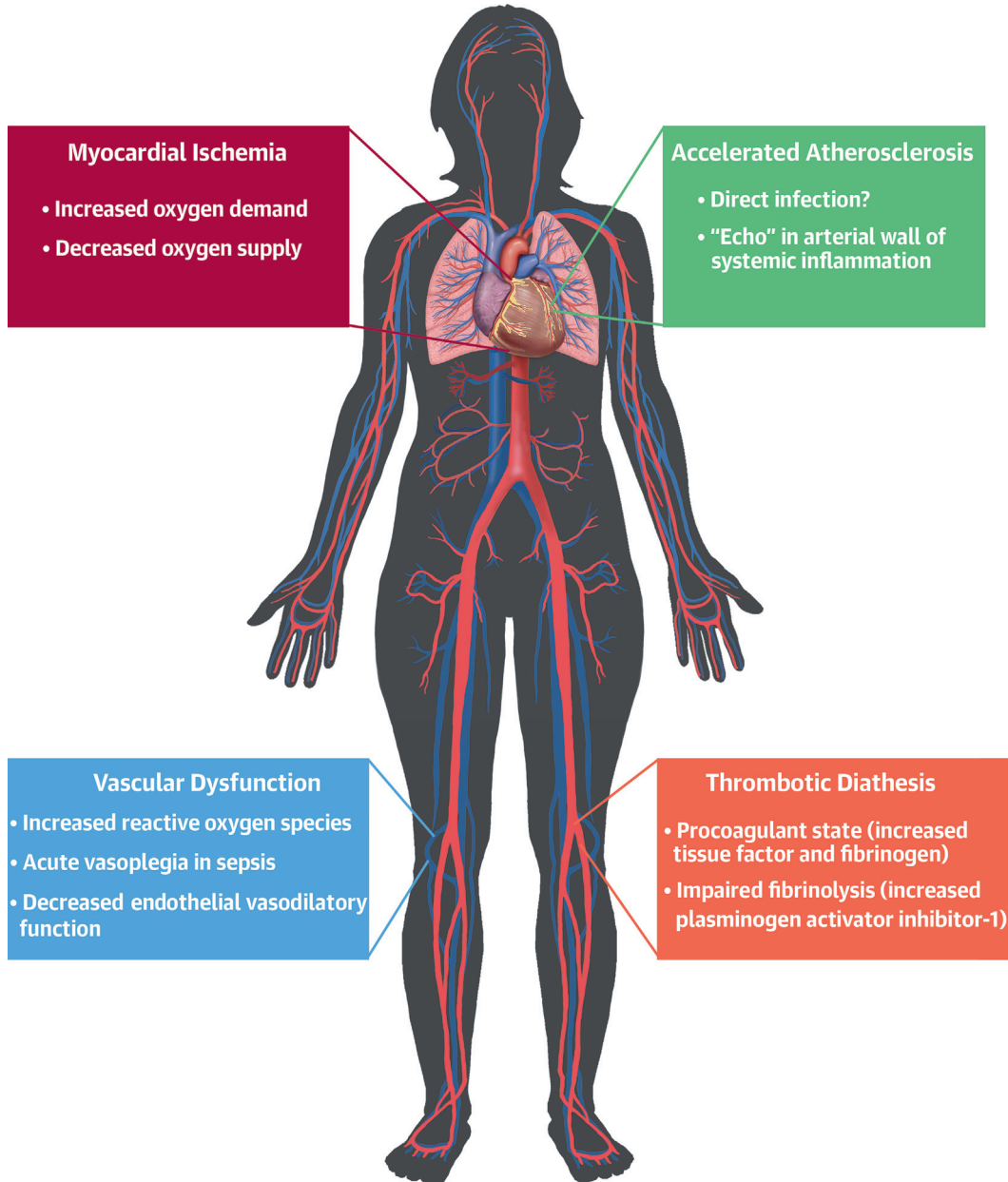


INFECTION AND ATHEROTHROMBOSIS: INDIRECT VERSUS DIRECT EFFECTS

Although direct microbial invasion does not seem to provide an actionable trigger for atherosclerosis, infection can still play a role in precipitating atherosclerotic events. Chronic infections in extravascular locations could provide a smoldering stimulus that contributes to inflammatory burden (71). Examples include periodontitis, bronchitis, urinary tract infection, infected cutaneous ulcers in patients with peripheral arterial disease, and diabetes mellitus. Infection at these sites could all provide niduses of inflammation remote from arteries that could evoke a systemic response that could accelerate

atherogenesis, reflected, for example, in modest elevations of C-reactive protein (Figure 2) (72-74). *C. pneumoniae* and certain microbial agents associated with periodontal disease in particular can elicit innate immune responses, aggravate experimental atherosclerosis, and associate with cardiovascular events (75-79). Azithromycin in particular concentrates in mononuclear phagocytes, the likely host to intracellular organisms such as *Chlamydia* species in the atheroma. However, antibiotic treatment may not effectively treat bacterial periodontitis because of an inability to penetrate the biofilms elaborated by oral microorganisms. *Helicobacter pylori*, a bacterium associated with gastric and duodenal ulcer disease, does not affect atherosclerosis in

CENTRAL ILLUSTRATION Acute and Chronic and Direct and Indirect Effects of Infection Can Augment Cardiovascular Risk



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The **left side** of this diagram depicts the effects of infections, generally acute, on aggravating myocardial ischemia (as detailed in [Figure 3](#)) and on producing vascular dysfunction in systemic and coronary arteries that can favor the development of cardiovascular events. The **right side** of the figure portrays how direct infection or echoes in the arterial wall of systemic or remote inflammation (as depicted in detail in [Figures 1 and 2](#)) can accelerate atherosclerosis. Both acute and chronic infections can heighten thrombotic risk by inducing a procoagulant state; for example, increasing tissue factor procoagulant production and fibrinogen elaboration by the hepatocyte (as shown in [Figure 2](#)) and impairing fibrinolysis by augmenting the expression of plasminogen activator inhibitor-1 as part of the acute-phase response. Thus, both acute and chronic infections can influence cardiovascular risk and infections remote from the artery, or potentially within the artery, and can thereby lead to augmented risk of cardiovascular events.

hypercholesterolemic mice, indicating some selectivity in microbial potentiation of traditional risk factors (80).

In some instances, bacterial products seeded into the systemic circulation from remote sites of infection could trigger a wave of acute inflammation by inflammatory cells as well as by products of native vascular cells that populate arterial lesions (81). We have dubbed this phenomenon the “echo” effect (Figures 1 and 2) (41). The increase in cardiovascular events and in thromboses in patients with pneumonia illustrates how remote infections can affect such outcomes (82). Experimentally, the local arterial inflammatory response to a systemic inflammatory stimulus derived from infectious organisms such as bacterial endotoxins can provide a greater local response in atherosclerotic arteries than in normal arteries (81). An example of such a stimulus is urosepsis, with leakage of endotoxin into the circulation from a bacterial source in the genitourinary tract.

We have recently gained an immense appreciation of the importance of the intestinal microflora in cardiometabolic disease (83,84). The astounding accumulation of bacteria and their products in the gastrointestinal tract provides a rich source of bacterial products such as endotoxins and heat-shock proteins, among others. In cases of impaired epithelial barrier function, bacterial products can leak into the circulation and provide another source of inflammatory stimuli that impinge on leukocytes lying in wait in atheromata or endothelial cells at sites of lesion formation. Such microbial products, pathogen-associated molecular patterns (PAMPs), activate innate immune receptors such as the toll-like receptors (TLRs) and provide stimuli for the activation of leukocytes and arterial cells within atheromata (Figure 2). Other microbial products such as trimethylamine oxide may also potentiate atherogenesis, as suggested by some, but not all, experimental and human observations (84,85).

Some stimuli derived from microorganisms can be strong (e.g., endotoxin engaging TLR4). Other stimuli can provide more subtle activation through the engagement of other innate immune receptors such as TLR2. Peptidoglycans can also serve as PAMPs (86,87). Other examples of bacterial PAMPs include lipoteichoic acid, which engages TLR2; viral and bacterial DNA, which can engage TLR9; peptidoglycans, which can engage TLR2; and mannans and beta-glucans associated with fungi, which can activate TLR2 and TLR4. PAMPs can also activate the nucleotide-binding and oligomerization domain-like receptors involved in the inflammasome that convert pro-IL-1-beta and pro-IL-18 into the active cytokines (88-91). Thus, although

the direct infection of the arterial lesion may not commonly drive atherogenesis, products of bacteria and other microorganisms in remote sites may elicit “echoes” from cells within lesions on both an acute or a chronic smoldering basis in a manner that can promote the evolution of lesions and their complication.

The responses to infection may also precipitate acute complications of atherosclerosis or amplify their consequences. For example, during sepsis, tachycardia and fever can lead to a hyperkinetic state that increases oxygen demands and predisposes to type 2 acute coronary syndromes (Figure 3) (56). Decreased oxygen supply due to hypertension and hypoxemia during sepsis can aggravate ischemic injury to the myocardium. In addition, the acute-phase reactants fibrinogen and plasminogen-activator inhibitor can promote thrombosis and impede endogenous fibrinolysis (Figure 2). Thus, although PAMPs can promote atherosclerotic lesion evolution generally in a chronic manner, the acute consequences of bacterial infections can augment myocardial oxygen requirements, decrease oxygen availability, promote clot formation, and impair the ability of the endogenous fibrinolytic system to clear thrombi when they form. Indeed, considerable recent observational and pathophysiological evidence supports the association between recent respiratory infections (92-96) or influenza (97) and atherosclerotic events. Indeed, large clinical trials are currently investigating the proposition that influenza vaccination can forestall cardiovascular events (98,99). Limiting influenza infections in vulnerable populations could remove a stimulus that elicits the inflammatory “echo” of systemic inflammation in atheromata (Figures 1 and 2) as well as the increased “demand” that can precipitate events (Figure 3).

CONCLUSIONS

Incontrovertible evidence now supports the importance of innate and adaptive immune pathways in atherogenesis. Moreover, infections may influence this disease process acutely, chronically, and directly or indirectly (Central Illustration). Although modulation of adaptive immunity has not yet reached clinical maturity as a therapeutic target, a number of strategies for vaccination are being investigated (100). The refinement of vaccination therapies for atherosclerosis in experimental studies continues to sustain interest in the clinical application of this approach (101). Targeting of innate immunity is reaching clinical maturity, with large-scale trials evaluating low-dose colchicine and anticytokine strategies in patients at risk for atherosclerotic events (102-106).

CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) showed that reducing inflammation by administering an anti-IL-1 beta antibody in men and women who had had a previous heart attack and residual inflammation despite standard-of-care therapy can reduce recurrent events (107,108). The anti-inflammatory therapy yielded a significant 15% reduction in the primary endpoint of “hard” major adverse cardiovascular events.

Biomarkers of the innate immune response, notably C-reactive protein (measured with a highly sensitive assay [hsCRP]), have entered clinical practice and have proven utility in targeting therapies to patients in ways that can effectively reduce their risk (109,110). In the JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) primary prevention study, statin therapy was allocated on the basis of an hsCRP value above the median for the population (>2 mg/l) but with an LDL level <130 mg/dl. The statin-treated group had a 44% reduction in first-ever cardiovascular events. In secondary prevention, CANTOS allocated the anti-IL-1 antibody to patients with stable post-acute coronary syndromes who had an hsCRP value >2 mg/l despite effective statin therapy. Those CANTOS participants who achieved a reduction of hsCRP to <2 mg/l in response to the anti-inflammatory therapy had a >30% reduction in cardiovascular and all-cause mortality (111). These examples illustrate the potential of guiding therapy

according to inflammatory status assessed by biomarkers in trials and in clinical practice. Contemporary human genetic studies provide strong support for innate immune pathways in atherogenesis. For example, Mendelian randomization studies implicate the IL-6 pathway as a causal participant in the generation of atherosclerotic events (112,113).

Although direct infection may not be a common driver of atherogenesis, remote infections and bacterial products from extra-arterial infections or colonization may promote atherosclerosis on a chronic ongoing or episodic basis (Figure 2). Acute catastrophic bacterial infections such as Gram-negative sepsis can precipitate type 2 acute coronary syndromes (Figure 3). The decades of research into inflammatory and immune pathways implicated in atherosclerosis and its complications have begun to yield biomarkers that inform clinical practice. Further exploration of these pathways may identify new therapeutic targets to address the unacceptable burden of residual risk that persists beyond the management of traditional risk factors.

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KEY WORDS basic & translational research