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An Unsettled Debate About the Potential Role of Infection in Pathogenesis of Atherosclerosis

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Abstract

Association of infection with atherosclerosis is by no means new. Several sero-epidemiological and pathologic studies as well as animal models have shown a link between infection and atherosclerosis. Exciting discoveries in recent times related to role of inter-individual genetic variation in modulating inflammatory response to infection have reignited the enthusiasm in proving a causal link between infection and atherosclerosis. The purpose of this article was to review and analyze the available evidence linking infection with atherosclerosis.

Keywords: Multifactorial; Inflammatory; Antibiotics

Introduction

Atherosclerosis is a multifactorial disease with several clinical manifestations like coronary artery disease (CAD), acute myocardial infarction (MI) and stroke [1]. Role of traditional risk factors like age, sex, hypertension (HTN), diabetes mellitus (DM), smoking and dyslipidemia in atherosclerosis has been well established [2]. Cumulatively, these risk factors have a better ability to predict future risk than any single risk factor but they are not sufficient enough to explain the total burden of cardiovascular (CV) heart disease in the population [2-5]. Many human as well as animal models studies have shown relation between infection and atherosclerosis [6]. Infectious agents by evoking cellular and molecular changes provide a major stimulus for initiation and propagation of inflammation, an important mechanism involved in atherosclerosis [7-9]. Although negative outcomes of antibiotics trials have raised the question of role of infection as an inflammatory stimulus in pathophysiology of atherosclerosis, infection hypothesis continues to fascinate researchers because instead of ruling out the role of traditional risk factors, infections act through or in concert with them [10, 11].

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Mechanism of Atherosclerosis

Atherosclerosis is a disease of blood vessels where both innate and adaptive immune systems are involved and inflammation plays a crucial role at all stages of it [6, 9, 12, 13]. Histologically, normal arteries have trilaminar structure: tunica intima (single layered endothelium with its basement membrane and subendothelial space), tunica media (smooth muscle cells) and tunica adventitia (mast cells, nerve endings and microvessels) [14]. A primary initiating event in atherosclerosis is accumulation of low-density lipoprotein (LDL) after oxidative modification in subendothelial space which stimulates overlying endothelial cells to produce adhesion molecules, chemotactic proteins like monocyte chemotactic protein-1 (MCP-1) and growth factor like macrophage colony-stimulating factor (M-CSF) resulting in migration of monocytes into subendothelial space [12, 15]. Monocytes differentiate into macrophages which uptake oxidized LDL, a process mediated by a number of scavenger receptors like SR-A and CD36, leading to formation of foam cells which are the building blocks of fatty streaks [16-18]. Fatty streaks could progress into atherosclerotic plaque which is composed of two elements: a small lipid core composed of modified lipids, macrophages and T cells and a fibrous cap made up of vascular smooth muscles, smooth muscle cells derived extracellular matrix and inflammatory cells like macrophage foam cells [12, 19]. These atherosclerotic plaques could either erode or rupture causing thrombus formation and leading to acute coronary events [18, 19]. Plaque rupture is mainly dependent on size of lipid rich core, thickness of fibrous cap and inflammation within the cap [19, 20].

Many cytokines such as interleukin (IL)-1, IL-6, IL-10, IFN-Y and TNF-Y are expressed highly in atherosclerotic lesions and an imbalance between pro- and inflammatory cytokines is known to be associated with atherosclerotic plaque growth and rupture [9, 21]. High plasma levels of IFN-Y and TNF are associated with development of unstable plaques susceptible to rupture, whereas a high level of IL-10 has strong anti-inflammatory and atheroprotective effects [9, 17, 22, 23]. Discovery of a critical role played by Toll receptors in onset

and progression of atherosclerosis has been an important step in unraveling molecular mechanism of atherosclerosis [24]. NF-kB activation is essential in regulating different genes involved in inflammation and proliferation of cells critical to atherogenesis [25]. Toll like receptor and IL-1R-associated kinase (TLR- IRAK) signaling pathways by activating NF-kB induce transcription of inflammatory cytokine and chemokine genes critical to initiation and progression of atherosclerosis [26].

Infection, Molecular Mimicry and Atherosclerosis

There is ample evidence to suggest that infection-induced immune-mediated reactions contribute to atherosclerosis [27]. Molecular mimicry, a host immune response initiated against invading pathogens with significant homology to host proteins cross-reacting with host structures, has been proposed to explain the link between infection and atherosclerosis [28]. Studies identifying autoantibodies to heat shock proteins (HSPs), a group of intracellular proteins whose expression to cellular surface of vascular endothelial cells increases in response to stress, in patients with atherosclerosis have increased the importance of molecular mimicry [29-31]. Several micro-organisms (*Chlamydia pneumoniae* (Cpn), *E. coli*, and mycobacteria) express homologus forms of HSPs [32]. These stress proteins acting as key target antigens recruit T lymphocytes from general circulation to vessel wall and by transforming Th1 dominant T lymphocytes contribute to foam cell formation [27, 31, 33].

Infection Has Been Linked to Atherosclerosis at an Early Age

Role of acute infections in producing coronary intimal thickenings were observed in autopsied older infants and children [34, 35]. Healthy children with persistent antibodies against Cpn were found to have an increase in intima media thickness (probably reflecting increased fatty streak formation) suggesting a role of infection in early atherosclerosis [36]. Similarly, in a longitudinal population-based study done in Australia, severe childhood infection was associated with subsequent hospitalization in adulthood from ischemic heart disease (IHD), ischemic stroke and peripheral vascular disease independent of population level risk factors further strengthening the role of infection in atherosclerosis and CV heart disease [37].

Acute Infections and Atherosclerosis/CV Disease

Acute infections by inducing inflammation affect atherosclerotic disease [38]. Studies have associated acute respiratory tract infection, urinary tract infection and bacteremia with acute coronary syndrome (ACS) [39-44]. This association is strongest for acute respiratory tract infection, in particular influenza, and our discussion will be limited to it.

Influenza

Several studies (mainly retrospective and one prospective) have suggested that acute respiratory infections can trigger acute MI [45-48]. It is now widely recognized that influenza epidemics are responsible for an increase in acute MI and excess deaths from IHD [49]. Autopsy study has proved this association [50]. Observational studies with the use of oseltamivir in patients with history of CV disease infected with influenza have shown a reduction in subsequent acute cardiac events [51]. These observations and studies make association of influenza with ACS fascinating.

In order to explain the mechanism by which influenza virus causes atherosclerosis, animal models have been created. In lipoprotein E-deficient (apoE$^{-/-}$) mouse model, a standard model to study atherosclerosis, influenza infection induced heavy infiltration of atherosclerotic plaques by inflammatory cells as well as fibrin deposition and thrombus formation [52]. Similarly, following inoculation with influenza, influenza viral antigens were detected in atherosclerotic plaque among apoE-deficient mice but not in non-virus inoculated control animals [53]. Viable influenza virus was recovered from aorta of normal and atherosclerotic mice in absence of viremia after infection with influenza indicating contribution of influenza infection to atherosclerosis [53]. Influenza virus is also known to cause a prothrombotic state by increasing thrombin generation and destabilization of vulnerable atherosclerotic plaque by increasing number of proinflammatory and prothrombotic cytokines [54, 55]. High levels of inflammatory cytokines and chemokines in plasma were found in patients with influenza A (H7N9) and H5N91 and they co-related with severe clinical outcomes including death [56, 57]. Finally, influenza vaccine was found to reduce major CV events in patients with ACS [58, 59]. Successful outcomes of influenza vaccination for CV patients were recognized by AHA and ACC and influenza vaccination has been recommended in their guidelines as a part of secondary prevention for CV disease [60]. In animal model of mice, influenza vaccine was found to stabilize atherosclerotic plaque by lowering pro-inflammatory markers while favoring synthesis of anti-inflammatory cytokine IL-4 [61].

Chronic Infections and Atherosclerosis

The hypothesis that infectious agents are associated with development of atherosclerosis gained traction when Fabricant et al showed that Marek's disease virus, a chicken herpes virus, could induce atherosclerosis in chickens [62]. A wide variety of pathogens, both viruses and bacteria, have been identified as factors leading to a chronic inflammatory state and contributing to atherosclerosis. Most of these pathogens have some common features like being intracellular pathogens and able to establish persistent infection [63]. Evidence on involvement of these pathogens in atherosclerosis has been based on sero-epidemiologic studies, detection of pathogens DNA in atherosclerotic plaque, isolation of pathogens from atherosclerotic lesions and *in vitro*/*in vivo* studies demonstrating direct role of pathogens at various stages of atherosclerosis. Among these

pathogens, cytomegalovirus (CMV) and Cpn have been studied extensively and we will review the available data on them including pathogenetic mechanisms.

CMV

Strong evidence for association of CMV with native coronary vessel atherosclerosis comes from a prospective epidemiological study, the ARIC study, where high levels of antibody to CMV were significantly associated with incident CV disease [64]. During 9 years of follow-up in the elderly Latino community, high CMV I_gG antibody titers which correlated with elevated TNF-alpha and IL-6 levels were associated with increased CV deaths suggesting a relationship between ongoing inflammation, high CMV titers and observed mortality [65]. Elevated CMV antibody titers were associated with an increased risk of carotid intimal-medial thickening, a measure of subclinical atherosclerosis after 13 - 18 years in one longitudinal prospective study [66]. CMV seropositivity was also found to be an important predictor of coronary restenosis after coronary angioplasty. Forty-three percent of CMV seropositive patients had restenosis at 6 months compared with 8% of CMV seronegative patients [67]. In the realm of transplantation, an increased rate of atherosclerotic events was observed in patients with post-transplant CMV replication compared to those without replication [68]. Several other sero-epidemiological studies have also linked CMV infection with post-transplant atherosclerosis [69, 70]. Not all studies, however, show a positive association between CMV seropositivity and CAD. In a large cohort of nearly 900 successive non-transplant patients undergoing coronary angiography, CMV infection was not found to be a major risk factor for development of primary CAD defined as greater than 50% of blockage in any coronary arteries [71]. Since the control group in this study comprised of patients with clinical indication for angiography, many of them who were found to have positive antibodies to CMV probably had some atherosclerosis. Some other prospective studies evaluating CMV seropositivity and risk of future CV events (including MI and death) in middle-aged individuals and older adults have failed to show a positive association [72-74]. A careful analysis of these studies, however, reveals that CMV titers had not been measured quantitatively and CMV seropositivity was used solely as a dichotomous variable [75]. Quantification of antibody titer could be important since one of the cross-sectional studies done in young aged individuals found that seropositivity to CMV in itself was not associated with risk of premature MI but a titer of anti-CMV I_gG greater than 100 EU/mL was associated with an increased risk of premature MI [76]. Since different studies utilize different clinical outcomes along the spectrum of disease progression and severity, a great amount of caution should be needed while interpreting and comparing the results of different studies [75].

Isolation of CMV From Atherosclerotic Plaque

If CMV were to be considered in the pathogenesis of atherosclerosis, a vascular tropism for CMV at sites prone for atherosclerotic lesions needs to be demonstrated. But the available studies on the frequency of CMV DNA in human atherosclerotic plaques have become confusing. While some studies show CMV DNA to be highly prevalent, others could not detect any CMV DNA [77]. A study done by Hendrix and his colleagues found that in CMV seropositive patients, CMV nucleic acids were equally distributed among arteries with and without atherosclerotic changes [78]. Recent studies however have identified CMV DNA in atherosclerotic plaques and not in non-atherosclerotic vessels [77, 79].

Animal Models for CMV

To study the role of infections in atherosclerotic process, animal models are a necessity. ApoE participates in hepatic clearance of cholesterol rich LDL from plasma and when it is knocked out in mice they acquire high plasma cholesterol levels and demonstrate progressive series of atherogenic events similar to humans making this model suitable for studying genetic and environmental influences on atherosclerotic process [80]. Chronic infection of apoE knockout mice with murine CMV increased atherosclerotic lesion size by both direct (increased production of pro-inflammatory cytokines and presence of murine CMV DNA in the lesion) and indirect effects (increasing systemic pro-inflammatory cytokines) [81]. Even injection of non-infectious virus (ultraviolet inactivation of murine CMV) caused an acceleration of atherosclerotic lesions in apoE knockout mice suggesting a possible immune-mediated aggravation of atherosclerosis [82]. Some of the mechanisms involved in initiation and progression of atherosclerotic disease by CMV include inflammatory cell influx (mainly T lymphocytes), intimal thickening by stimulating smooth muscle cell proliferation and increased aortic expression of pro-atherosclerotic genes selectively recruiting monocytes by up-regulating chemokines (MCP-1 expression) [83-85].

Cpn

Cpn is probably one of the most extensively studied micro-organisms in an effort to prove association of infections with atherosclerosis. Saikku et al have reported an association between Cpn infection and coronary atherosclerosis for the first time in 1988 [86]. Since then there have been multiple sero-epidemiologic studies with contradictory results. The studies were done in different populations using different criteria for cases or various cutoff titers to define seropositivity and have been adjusted for potential confounders to varying degrees making them susceptible to different kinds of biases [87]. One meta-analysis of 15 prospective trials evaluating I_gG titers involving over 3,000 cases and over 9,000 controls found no significant association between CAD and I_gG titers [88]. Another meta-analysis of 10 prospective trials evaluating I_gA titers involving greater than 2,000 cases and over 7,000 controls found a significant but weak association with I_gA titers [89]. Cpn was detected in 50-62% of atheromatous plaques from symptomatic

patients but was rarely detected in non-atherosclerotic tissues [90, 91]. Viable chlamydiae have been isolated from primary atheromatous lesions as well as from secondary restenotic by pass lesions [92]. They were detected by electron microscopy in coronary artery fatty streaks and atheromatous plaques in autopsies [93]. Recently, higher density of Cpn cells was found to be more prevalent in plaques associated with ACS [94]. But the debate regarding whether they were causative pathogens or innocent bystanders persists. Etiologic significance of presence of Cpn in atheromatous lesions can be ascertained only after demonstrating its capability of either initiating or potentiating atherosclerosis in *in vitro* and *in vivo* studies. Based on *in vitro* studies, chlamydia was found to have the ability to infect, proliferate and maintain its infectivity in cells that are common components of atherosclerotic plaque like endothelial cells, macrophages and smooth muscle cells [95, 96]. Cpn infection of cultured endothelial cells activates transcription factor NF-kB upregulating expression of several genes causing cell proliferation, inflammation and progression of atherosclerosis [97, 98]. Chlamydia is also known to cause production of large amounts of chlamydial HSP60 (cHSP60) during chronic persistent infections [9]. cHSP60 by inducing TNF-alpha and MMP expression by macrophages increases endothelial expression of E-selectin, ICAM-1, VACM-1 and IL-6, whereas by activating NF-kB complexes it could contribute to atherogenesis and plaque instability [99, 100]. HSP 60 of Cpn by inducing lectin like oxidized LDL receptor 1 (LOX-1) is known to mediate fatty streak formation in hypercholesterolemic rabbits [101]. In animal models like atherosclerosis prone apoE$^{-/-}$ mice, chlamydia infection was able to result in acceleration of atherosclerosis [102]. Finally, chlamydia possesses ligands for both TLR4 and TLR2 and blocking them may have a beneficial effect on atherosclerosis [103]. All of these findings were convincing enough to suggest a strong association of Cpn with atherosclerosis/CV disease and this has led to the designing of several antibiotics trial in a hope of improving CV outcomes in CAD patients. Four large-scale randomized trials (WIZARD, ACES, CLARICOR and PROVE IT-TIMI) had studied the effects of antibiotics in prevention of composite CV events. Unfortunately, the results of these trials have been discouraging. A meta-analysis of antibiotic trials has failed to detect a significant association between antibiotic use and secondary prevention of composite CV events [104]. The negative results of these trials have dampened the enthusiasm in associating infections with atherosclerosis and its complications [105]. But there are a number of things that need to be considered while interpreting the results of these antibiotic trials. First, the trials were secondary prevention studies and antibiotics will not be able to reverse or modify established pathology of advanced atherosclerosis [106, 107]. Second, if infection were to play a role in atherogenesis and its complications, pathogen burden (aggregate pathogens including viruses and bacteria) would best correlate with this role and the chosen antibiotic might not be able to eradicate all the infections [105]. Lastly, given the role of complex immune-related and genetically manipulated mechanism in initiation and progression of atherosclerosis, antimicrobials combined with immunosuppressive medications as in other immune-mediated infection-induced complications like post-infectious glomerulonephritis and Guillain-Barre syndrome might achieve significant benefit than antibiotics alone [63].

Inflammatory and immune response modulating atherogenic effects of infection can vary based on genetic differences [63]. When mice with genetic deficiency of apoE was infected with Cpn, accelerated atherosclerosis and increased serum levels of pro-inflammatory cytokines were observed but when they had additional genetic deficiency in TLR2 and TLR4, atherosclerotic plaque development was significantly inhibited [102]. Patients with IL-1 gene polymorphism and Cpn seropositivity were found to be more likely to develop CAD than seropositive patients without IL-1 gene polymorphism [108]. Further research into the role of genetic variation in modulating the atherogenic effects of infection could be instrumental in clarifying the association between infection with atherosclerosis.

Pathogen Burden and Atherosclerosis

The concept of pathogen burden which means cumulative burden of infection rather than one specific infection is an emerging one in the pathogenesis of atherosclerosis [109, 110]. Zhu et al first demonstrated that increasing pathogen burden instead of an individual pathogen contributes to atherosclerosis. CAD prevalence was 77% higher in the group with antibodies to five pathogens (CMV, hepatitis A virus, Cpn, herpes simplex virus-1 and herpes simplex virus-2) compared with the group with antibodies to two or less than two pathogens [111]. In a prospective study, researchers studied impact of pathogen burden on long-term prognosis in patients with CAD and found that aggregate number of pathogens was more predictive of future fatal CV events [112]. Simultaneous detection of two pathogens in atherosclerotic lesions and demonstration of synergistic effect of different pathogens on expression of atherosclerotic factors like IL-6, IL-8, etc, in vascular smooth muscle cells involved in formation of atherosclerotic plaque further support the hypothesis of pathogen burden [113, 114]. Role of pathogen burden in the pathogenesis of atherosclerosis has probably been underestimated and further research will be needed to clarify this complex association [115].

Summary

Based on the available evidence, there is no doubt that infections do play a role in atherosclerosis and its complications. How infections play a role in atherosclerosis and at what point of its natural history is still controversial. Given the complex nature of atherosclerosis initiation and progression, even when infections were found to be causally related to atherosclerosis, whether eradicating them could result in significant benefit in terms of clinical outcome is yet to be determined. More research in the years ahead is needed to clarify the role of infection in atherosclerosis.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Udip Dahal made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. Dikshya Sharma was involved in drafting and revising the manuscript. Kumud Dahal made the final approval of the version to be published.

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