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REVIEW ARTICLE

The Role of Microbiologic Agents in the Progression of the Atherosclerosis: a Comprehensive Review

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Abstract

Atherosclerosis is a leading cause of disability, morbidity and mortality in the world. Atherosclerosis is accepted as a chronic progressive inflammatory disease. The inflammatory cascade in the vascular wall is well-defined. However, the predictors and contributors of the inflammatory response in atherosclerosis are not completely understood. Systemic and local inflammation, which enhance the burden of inflammation in the vascular wall, have been proposed as risk factors for the progression of atherosclerosis. Infectious micro-organisms are one of the major triggering factors for local and systemic inflammation. In this review, we aimed to emphasize the linkage between micro-organisms and the progression of atherosclerosis. We briefly summarize the current medical literature and discuss the future perspectives of the linkage between microbial agents and atherosclerosis representing cause and effect.

Keywords: Atherosclerosis, Inflammation, Vascular disease, Micro-organism, Microbiota

1. Introduction

Atherosclerosis is accepted as a chronic and progressive inflammatory disease [1]. The predictors and contributors of atherosclerosis are multifactorial. Inflammation plays a central role in the initiation and progression of atherosclerosis [2]. In this review, we aimed to emphasize the role of microbial agents in the progression and complications of the atherosclerotic process. Initially, we summarize the early studies that showed the direct isolation of microbial agents from atherosclerotic plaques. Then, we will focus on gastrointestinal flora and discuss the impact of the oral and gut microbiome in the progression of atherosclerosis. We will also discuss the treatment strategies for suppressing the inflammatory reaction in atherosclerosis. Finally, we will review the future perspectives on this topic.

2. Definition of Atherosclerosis

Atherosclerosis is defined as the accumulation of fatty and fibrous material in the intimal layer of the artery [1]. Atherosclerosis is a leading cause of morbidity and mortality all over the world. One third of all deaths occur secondary to arteriosclerosis and complications of atherosclerosis [3]. Involvement of the coronary vessels leads to acute coronary syndrome and myocardial infarction, which is a major killer worldwide. Involvement of the aorta and carotid vessels leads to a transient ischemic attack or stroke, which are leading causes of disability and mortality. Involvement of the peripheral vessels in the upper and lower extremities leads to claudication, ulceration and amputation. Moreover, the involvement of visceral vessels can lead to other important clinical syndromes.

Atherosclerosis has traditionally been accepted as the deposition of lipids within the vessel wall of medium-sized and large arteries [3]. Several investigations have been performed that showed the

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clinical and demographic parameters that affect the progression of atherosclerosis [3]. Nowadays, clinical and demographic predictors of atherosclerosis have been definitively reported in numerous studies [3]. Dyslipidemia, diabetes, hypertension, smoking, sedentary lifestyle, genetic predisposition, dietary factors and stress are major contributors to the atherosclerotic process [3]. Low density lipoprotein (LDL) cholesterol has been pointed out as a major contributor to atherosclerosis. A decrease in LDL cholesterol has been linked to fewer vascular events and also to slowing down the atherosclerotic process [3]. Nonetheless, effective cholesterol reduction is not enough to stop or reverse the atherosclerotic process. Further investigations have revealed that the pathophysiology of atherosclerosis is more complex, and the deposition of LDL cholesterol is only a single factor in the progression of atherosclerosis [4]. Recently, atherosclerosis was defined as chronic inflammation of the vessel wall [3–5]. It involves complex endothelial dysfunction induced by elevated LDL-cholesterol, free radicals, infectious microorganisms, shear stress, hypertension, toxins and a combination of these factors, which lead to a compensatory inflammatory response [3–5]. Moreover, rheumatic and autoimmune diseases that trigger the inflammatory process in the body may affect the development of atherosclerosis. Psoriasis, rheumatoid arthritis and inflammatory bowel disease are examples of chronic systemic inflammatory diseases that may accentuate the progression of atherosclerosis.

3. Inflammatory Cascades in the Progression of Atherosclerosis

The interection between lipids and immune activation is the hallmark of atherosclerosis. Both local and systemic inflammatory response get involved in the initiation and progression of atherosclerosis. The initial step in atherosclerosis is endothelial dysfunction characterized by decreased nitric oxide synthesis [6]. Shear stress, especially in the curvature and branching points of the vessel, are the major triggering factor for the initiation of atherosclerosis. Oxidative stress, free oxygen radicals, inflammatory mediators aggrevate the local effect of shear stress which initiate local inflammation. The next step is local oxidation of LDL-cholesterol and its accumulation in the vessel wall. Immune activation against LDL cholesterol initiates the systemic inflammatory response. The upregulation of cell adhesion molecules facilitates the adherence and transmigration of leukocytes into the vessel wall. T cells recognize oxidized LDL, heat shock protein and shared microbial antigens via molecular mimicry and locally release proinflammatory cytokines. The stimulation of macrophages by T-cell derived cytokines leads to the uptake of oxidized LDL and the formation of foam cells, a hallmark of the early atherosclerotic reaction. These cells secrete matrix metalloproteinases which lead to further progression of atherosclerosis [3,6].

The major drugs used in atherosclerosis, i.e. statins and renin-angiotensin inhibitors, partially mediate their effects by modulating the immune response and inflammation [3].

Abbreviations

LDL	Low density lipoprotein
DNA	Deoxyribonucleic acid
TMAO	Trimethylamine-N-oxide
CRP	C-reactive protein
TNF-α	Tumor necrosis factor α
IFN-γ	Interferon γ
IL-1	Interleukin 1
IL-6	Interleukin 6
Ig A	Immunoglobulin A
CMV	Cytomegalovirus
EBV	Epstein–Barr virus
HCV	Hepatitis C virus
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
NSAIDs	Non-steroidal anti-inflammatory drugs
IL 1β	Interleukin 1β
TLRs	toll-like receptors

4. Inflammation As an Etiologic Factor in Atherosclerosis

Inflammation in the atherosclerotic process was previously thought to be a microbe-free, sterile reaction. However, recent investigations have suggested the impact of microbial agents in the progression of atherosclerosis [2,5]. Several investigations have been performed to show the role of microbial agents in atherosclerosis, although the results were variable [7–10]. Early studies proposed that *Chlamydia pneumoniae*, *Helicobacter pylori* and *Mycoplasma pneumoniae* may have an impact on the progression of atherosclerosis [8,9]. Recent investigations have mainly focused on the gastro-intestinal system flora [11,12]. Moreover, certain types of dental flora have been isolated from atherosclerotic plaques [13]. The isolation of certain members of the intestinal flora has raised the questions about the role of the microbiota in the progression of atherosclerosis [12]. Indeed, microorganism colonization of atheroma may be a bystander phenomenon, rather than a causative linkage with the

disease. All the investigation just showed the presence of the microorganism within the atheroma plaques. Further in-vivo investigations may show the direct effect of the microorganism within the atheroma plaques more clearly. Nonetheless, the direct isolation of microbial agents from the atherosclerotic plaque is clinically very important step to clarify atherosclerotic process. This linkage may yield to the generation of different treatment methods for the atherosclerosis. The inflammatory reaction produced by microbial agents may trigger the initial step of the atherosclerotic process and may have a role in the acceleration and complications of the disease.

5. Pathophysiology of Microbial Agents and Linkage to Atherosclerosis

Both oral and gut microbiota species are the well-known etiologic agents of infective endocarditis. Thus, antibiotic prophylaxis was recommended for invasive procedures involving oral cavity and gastro-intestinal systems in the high-risk patients. Recent studies showed that there would be a linkage between atherosclerosis and microbial agents including oral and gut flora also. The exact mechanism of the hazardous effect of microbial agent in the atherosclerosis still is not clear. Microbial agents may influence the atherosclerotic process either in a direct or indirect manner [14–17]. The isolation of microbial DNA from atherosclerotic plaques supports the direct effect of microbes. Direct invasion of the vascular wall may initiate the inflammatory reaction. The invasion of atherosclerotic plaques with different microbial agents could lead to further progression of the disease. Direct invasion of vulnerable plaques after transient bacteremia may lead to the rupture of plaques, which leads to serious clinical events. Direct isolation of microbial agents was carried out both on coronary and carotid plaques [15,18]. Atherosclerotic progression in the coronary arteries and carotid arteries are a major site of complications and the occurrence of clinically relevant disease. In light of the current medical literature, we can conclude that the colonization of the coronary and carotid vascular wall by certain microbial agents may accelerate the disease and may lead to clinically relevant complications earlier. Indirect effects may become evident with the distant production of inflammatory markers. Analysis of atherosclerotic plaques has shown that there is abundant production of cytokines and inflammatory factors within plaques [15,16]. Microbial agents may increase the systemic inflammatory reaction with distant effects. Moreover, microbial agent also may have indirect effects via some metabolites. Recent studies have shown that accumulation of the pro-atherogenic factor trimethylamine-N-oxide (TMAO) is linked to intestinal trimethylamine (TMA) production by certain strains of gut microbes [16,19]. The gut flora also has an impact on cholesterol and glucose balance. The indirect effects of the gut microbiota seem to be more important than the direct invasive effect.

5.1. *Chlamydia pneumoniae*

Chlamydia pneumoniae is a common cause of respiratory tract infections. However, it has the ability to disseminate systematically and may cause chronic inflammation. It can easily disseminate through peripheral blood mononuclear cells and colonize extrapulmonary tissue, predominantly the vascular wall [7]. *C.pneumoniae* was the first microbial agent blamed for the progression of atherosclerosis. Long-term localization of *C. pneumoniae* in the vessel wall may trigger an inflammatory response, which may accelerate the atherosclerotic process. Initial investigations showed that there was a correlation between serologic positivity for *C. pneumoniae* and atherosclerotic vascular diseases [20]. Persistent infection with *C. pneumoniae* enhances the systemic inflammatory response, evidenced by high levels of C-reactive protein (CRP) and IL-6. In vivo studies showed that atherosclerotic lesions are exacerbated following *C. pneumoniae* inoculation in hyperlipidemic animal models [16]. Further investigations with the direct isolation of *C. pneumoniae* from the carotid and coronary atherosclerotic plaques ensured the direct effect of *C. pneumoniae* in the atherosclerotic process. Persistent infection with *C. pneumoniae* leads to the secretion of proinflammatory cytokines such as TNF-α, IFN-γ, IL-1, IL-6, adhesion molecules and reactive oxygen species. All of these pathways contribute to the local and systemic chronic inflammatory reaction responsible for the initiation, progression and complication of atherosclerotic plaques [20]. *C. pneumoniae* has been isolated from carotid plaques in over half of reported cases [18,20–22]. In addition, carotid plaques with *C. pneumoniae* infection are more likely to be complicated by acute cerebrovascular accidents [19]. It has also been reported that serum antibody levels for *C. pneumoniae* do not predict the location of the bacteria within carotid plaques. So, carotid plaque infection with *C. pneumoniae* may progress slowly and asymptomatically as a local infection. A meta-analysis by Filardo et al. concluded that *C. pneumoniae* infection may contribute to atherosclerotic cardiovascular disease

by enhancing the inflammatory state; particularly, seropositivity to *C. pneumoniae* IgA together with high sensitive CRP, fibrinogen and IL-6 may be predictive of atherosclerotic cardiovascular risk [7].

Although most studies proposed the impact of *Chlamydia pneumonia* in atherosclerosis, antibiotic trials for the eradication of *C. pneumonia* failed to reverse this connection. The limited ability of antibiotics to diffuse into atherosclerotic plaques might be one reason for this. Additionally, after triggering the local inflammatory reaction, *C. pneumoniae* may return to a dormant form, on which antibiotic therapy is useless. Finally, *C. pneumoniae* seems to be only one infectious agent in the progression of atherosclerosis. Recent studies concluded that there are many microbial agents linked to atherosclerosis [9]. So, even effective antibiotic treatment with the eradication of *C. pneumoniae* would not be enough the reverse the atherosclerotic process because of the polymicrobial involvement of atherosclerotic plaques.

After the correlation between *C. pneumoniae* and atherosclerosis was described, other microorganisms were investigated that could lead to potential chronic infections within the body. The most common pathogens proposed to have a relationship with atherosclerosis are indicated in Table 1.

5.2. Other bacterial species

H. pylori, a bacterium associated with stomach disease, has been reported as a predictor of atherosclerosis [9]. Similar to *C. pneumoniae*, a definitive effect of the eradication of *H.pylori* to reverse atherosclerotic process is not clear.

M. pneumoniae is another respiratory pathogen that tends to induce chronic disease with an atypical presentation. Long-term infection with *M.pneumoniae* may enhance the inflammatory response in the vascular wall [9].

5.3. Viruses

Some viral agents have also been reported as predictors of atherosclerosis. Cytomegalovirus (CMV), Epstein–Barr virus (EBV), hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV), enteroviruses and influenza A have been reported as possible predictors of atherosclerosis [23]. However, their impact on the progression of atherosclerosis is variable. Cytomegalovirus is associated with an increased risk of cardiac allograft vasculopathy and it is associated with transplant rejection. It was shown that CMV infection decreased the 10 year cardiac allograft vasculopathy survival. The major mechanism of the vasculopathy was proposed as immune-mediated injury followed by inflammation [24]. Subsequent smooth muscle cell migration from the vessel media to the intima and proliferation lead to intimal hyperplasia and vessel narrowing. Cytomegalovirus infection may play a role in the denovo atherosclerotic process by triggering similar pathway.

Besides bacteria and viruses, some protozoa and fungi have also been isolated from atherosclerotic plaques, which brings their impact up for discussion [25].

5.4. Oral microbiota

Recent studies have primarily focused on oral and gut microbiome and their effect on atherosclerosis [16,26–28]. There are more than 700 species of bacteria living in the oral flora, and most of them have not been cultivated. It is well-known that the oral flora may cause transient bacteremia after a dental procedure, which may represent clinical evidence in patients with valvular heart disease. Nonetheless, even simple tooth brushing may trigger transient bacteremia. Each bacteremia attack is a risk factor for a systemic inflammatory response. On the other hand, most oral bacteria have poor virulence and do not trigger a systemic inflammatory response. However, they are still capable of producing a local inflammatory reaction. Transmission of such bacteria to the vascular wall and atherosclerotic plaques may affect the atherosclerotic process. Recent medical data also support this hypothesis [28–30]. The majority of carotid plaques involve more than a single bacterium from the oral microbiota, even when systemic inflammatory markers are normal [31,32]. More frequent bacteremia periods may be associated with an accelerated atherosclerotic process. Moreover, they may trigger plaque rupture and serious clinical circumstances during bacteremia. In each bacteremia episode, different species may settle within the atherosclerotic plaque and trigger a further local inflammatory reaction. So, the isolation of bacteria within plaques does not give information about the timing of bacteremia and infection. In such patients, hygiene of the oral cavity is very important. However, studies have shown that only certain species have a connection with atherosclerosis.

Porphyromonas gingivalis was a well-known oral microbe that has been proposed as a risk factor for atherosclerosis [13]. Many studies have confirmed the role of the oral microbiota in the atherosclerotic process [33–36]. Aggregatibacter, Proteobacteria, Actinobacteria, Chryseomonas, Veillonella,

Table 1. List of micro-organism that proposed have linkage in the progression of atherosclerosis.

Bacterias	Viruses:
Chlamydia pneumoniae	Ebstein-Barr Virus (EBV)
Helicobacter pylori	Cytomegaly virus (CMV)
Borrelia burgdorferi	Hepatitis B virus (HBV)
Pseudomonas aeroginosa	Hepatitis C virus (HCV)
Mycoplasma pneumoniae	Human immune deficiency virus (HIV)
Oral plaque microbiota (multiple strains)	Enterovirus
Gut microbiota (multiple strains)	Influenza A virus
	Herpes simplex virus 1 (HSV 1)
Protozoa:	Fungi:
Perkinsus qugwadi	Funneliformis mosseae

Streptococcus, Tannerella, Eikinella, Fusobacterium and Campylobacter are some of the microbes that have been isolated from atherosclerotic plaques [37,38] (Table 2). Analyses with more complex and specific techniques like pyrosequencing and next generation DNA sequencing have led to the isolation and even discovery of new species within atherosclerotic plaques [39–41]. Simultaneous analysis of periodontal biofilm and carotid plaques also provides evidence for role of the oral microbiome in the atherosclerotic process. Nowadays, it is accepted that chronic periodontal disease has a strong impact on the progression of atherosclerosis [33–38]. Besides the additional impact on the systemic inflammatory response, it may have a direct effect on the vascular wall after translocation during bacteremia.

The isolation of polymicrobial agents from atherosclerotic plaques in all cases supports their role in the symptomatology of the disease. Fak et al. reported that patients with symptomatic atherosclerosis had a higher relative abundance of Anaeroglobus than the control group [26]. Especially Parvimonas positivity within plaques indicates a higher risk of plaque rupture and clinical manifestations. Range et al. reported that carotid plaques involving *Tannerella forsythia* showed more prominent neutrophil activation and tended to complicate more frequently [42]. On the other hand, Lindskog et al. examined the different regions of the complicated carotid plaque in term of microbiome involvement [10]. They found that the majority of plaques involved Proteobacteria and Actinobacteria and bacterial numbers were not statistically different according to the symptomatology and region of the plaques. Although each study provided variable results in term of symptomatology and microbiologic involvement in the atherosclerotic plaque, it is obviously clear that there is a strong correlation between atherosclerosis and the oral microbiota.

5.5. Gut microbiota

The gut microbiota includes a large variety of microbiological species. Older traditional data have suggested that they play only a minimal role in the digestion process, while their metabolic effects are unknown. After the completion of the Human Genome Project, numerous investigations showed the effect of the gut microbiota in systemic events [14–17]. These studies confirmed that the gut microbiota has a direct effect on several systemic diseases like obesity, diabetes mellitus, hypertension, coronary heart disease and some auto-immune diseases. In the light of these studies, the gut microbiota was defined as a second brain, and clinical trials are still ongoing. Parallel to evidence of

Table 2. Periodontal pathogens which proposed have a impact in the progression of atherosclerosis.

Most common oral pathogens	Most common bacterial species
Anaeroglobus	Porphyromonas gingivalis
Firmicutes	Aggregatibacter actinomycetemcomitans
Proteobacteria	Tannerella forsythia
Actinobacteria	Eikenella corrodens
Chryseomonas	Fusobacterium nucleatum
Veillonella	Treponema denticola
Acinetobacter	Prevotella intermedia
Alloprevotella	Camplyobacter rectus
Fusobacterium	
Streptococcus	

a linkage between the oral microbiota and atherosclerosis, recent studies have shown that there is a close relationship between gut microbiota diversity and atherosclerosis [11].

The gut microbiota may have an impact on atherosclerosis either via direct invasion of atherosclerotic plaques or by modulating cholesterol metabolism and the production of harmful metabolites. Gut microbes producing TMA are considered harmful, whereas, bacteria producing propionic acid and butyrate are thought to be beneficial [43,44]. Moreover, gut bacteria have a direct effect on cholesterol metabolism. Gut bacteria may change the absorption of cholesterol and fatty acids, which may cause significant changes in cholesterol and triglyceride levels. The most common gut microbes associated with TMA accumulation are indicated in Table 3.

Table 3. Common gut microbes which associated with trimethylamine accumulation.

Anaerococcus hydrogenalis
Clostridium asparagiforme
Clostridium hayhewayi
Clostridium sporogenes
Edwardsiella tarda
Escherichia fergusonii
Proteus penneri
Providencia rettgeri
Providencia rustiganii

Trimethylamine-N-oxide is an end metabolic product of TMA. Recent studies have shown that an increased serum TMAO level is a predictor of cardiovascular disease. It promotes forward cholesterol transport, exacerbates impaired glucose tolerance and promotes adipose tissue inflammation. All these negative effects of TMAO have led to its definition as a proatherogenic factor [43]. Serum TMAO levels are dependent on gut TMA production from choline by the microbiota. Choline is necessary for a wide range of biological activities, including maintaining the structural integrity of cell membranes, supporting cholinergic neurotransmission and donating methyl groups in biosynthetic reactions. Choline availability depends on dietary intake. Excessive metabolism of choline by gut microbiota leads a deficiency of choline. Choline metabolism can vary according to the composition of the gut microbiota. Even with similar dietary habits, gut TMA production may differ according to the dominant species of the gut flora [43].

In vitro studies have shown that two major different phyla, *Firmicutes* and *Proteobacteria*, has the capacity to consume choline accumulate TMA. In contrast, *Bacteroidetes* colonization does not lead to TMA accumulation, so it can be defined as a beneficial microbe [43]. Recent studies have proposed that some anti-atherogenic molecules mediate their effects by modulating the gut microbiota. The beneficial effect of berberine and resveratrol on atherosclerosis are secondary to the modulation of the gut microbiome [19,45]. Studies performed in mice showed that both molecules mediate their effects by increasing the colonization of *Lactobacillus* and *Bifidobacterium*, which have a low capacity for TMA production. However, the majority of the gut microbiota is still not cultivated, and we do not know which phyla of the gut flora may have an effect on atherosclerosis. In each study, several new strains have been discovered that might have an impact on the progression of atherosclerosis. Ongoing studies and future investigations may show further linkages between the gut microbiota and atherosclerosis.

There is also linkage between heart failure and gut microbiota such as increased *Escherichia coli*, Klebsiella penumoniae, and Streptococcus viridans [46]. Barin JG showed that gut microbial dysbiosis may trigger autoimmune myocarditis and it may lead to subsequent maladaptive cardiac remodeling and heart failure. So, we can conclude that gut microbiota have a significant impact on the cardiac function by the distant immugenic effects [47].

6. Treatment strategies for the Inflammatory Aspects of Atherosclerosis

The basic approach to the treatment of atherosclerosis is primary prevention. The modification of risk factors including dyslipidemia, diabetes mellitus, hypertension and smoking is associated with a lower incidence of cardiovascular disease [3]. The major drugs used for secondary protection are lipid-lowering drugs, antiplatelet drugs and antihypertensive drugs predominantly influencing the renin-angiotensin system. Drugs that lower LDL levels are still the cornerstone of the treatment of atherosclerosis.

Vulnerable atherosclerotic plaques, defined as plaques with a lipid-rich core and a thin fibrous capsule, have a higher risk of complication. The rupture of vulnerable plaques can lead to vascular atherothrombosis, which is the major causes of myocardial infarction and stroke. There is an active inflammatory process within vulnerable plaques, and stabilization of the such plaques could mitigate clinical syndromes and lower mortality and morbidity rates [3]. The major drugs used for the stabilization of vulnerable plaques are statins and angiotensin receptor inhibitors/angiotensin

receptor blockers. It has been proposed that they mediate their stabilizing effects by modulating the inflammatory cascade. The use of both drug groups is associated with lower systemic inflammatory markers. However, higher doses are necessary to observe anti-inflammatory effects, and their effect is relatively weak in terms of reversing the inflammatory cascade [3].

Inflammation plays a central role in the progression of atherosclerosis. Theoretically, suppressing this inflammatory reaction might reverse the atherosclerotic process [48]. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of local and systemic inflammation. They inhibit prostaglandin synthesis and may exert beneficial effects on vascular inflammation. Contrary to expectation, studies with NSAIDs are associated with a higher rate of cardiovascular events [48]. Inhibition of prostacyclin, which has an important preventive effect on platelet aggregation, counterbalances the probable beneficial anti-inflammatory effects of these drugs. So, it is obviously clear that NSAIDs are not effective at modulating the inflammatory aspects of atherosclerosis.

A novel target for the treatment of inflammation in atherosclerosis is blocking interleukin- 1β (IL-1β) release from neutrophils. IL-1β plays important roles in innate immunity and has an important effect in the initiation and complication of atherosclerosis [48,49]. The monoclonal antibody canakinumab, a selective IL-1β inhibitor, was reported as being effective at decreasing the rate of cardiovascular events after myocardial infarction [48]. However, the cost of this therapy is high, and further trials are necessary to show the effect on stable patients. Studies with cheaper drugs that affect IL-1β release showed variable results. IL-1β has an important role in innate immunity [50–52]. Methotrexate is an anti-inflammatory drug used for some autoimmune diseases. Methotrexate exerts its anti-inflammatory effects partially by the inhibition of IL-1β release. However, investigations with methotrexate did not show similar results as those with canakinumab. Inhibition of IL-1β production with low-dose methotrexate did not show any beneficial effect on cardiovascular outcomes [50]. However, therapy with a low dose of colchicine, another molecule that blocks IL-1β production, seemed to be beneficial and was associated with lower serum inflammatory markers and fewer cardiovascular events [51,52].

Another novel therapeutic target is the inhibition of toll-like receptors (TLRs), which play essential roles in innate immunity [53]. TLRs are pattern recognition receptors that mediate various signaling pathways in infectious and sterile inflammation. Some recent investigations have proposed that the inhibition of TLRs alleviates the severity of atherosclerosis to some extent by suppressing vascular inflammation and the formation of atherosclerotic plaques [53]. The anti-inflammatory effect of statins, particularly fluvastatin, also occur by the inhibition of TLR. However, we need more extensive investigations to elucidate the favorable effect of TLR inhibitors.

It is logical to postulate that the eradication of microbial agents that are assumed to have an effect on atherosclerosis could modulate the atherosclerotic process. To check the validity of this hypothesis, antibiotic therapy was investigated to assess the effect on atherosclerosis. These studies did not show any beneficial effects of antibiotics on cardiovascular events. In these studies, the composition of the microbiota was not analyzed. Antibiotics induced the dysbiosis of gut microbiota and may have reversed the beneficial effects of antibiotics. Nonetheless, early and effective treatment of *C. pneumoniae*, *M.pneumoniae*, *H.pylori* and other predefined bacteria may prevent the dissemination of such species and decrease the likelihood of vascular invasion.

Modulation of the oral flora is very important to treat the inflammatory causes of atherosclerosis. Regular oral examination may show the degree of periodontitis, which has been linked to more frequent cardiovascular events. Treatment of chronic periodontitis with antiseptic solutions and regular tooth-brushing may decrease the level of inflammation. Cultivation of the oral flora in advanced periodontitis patients and even antibiotic therapy for certain harmful species might have an impact on the systemic inflammatory response.

The role of the gut microbiota in the progression of atherosclerosis is more complex. An unhealthy diet and prolonged antibiotic treatment may lead to dysbiosis and the accumulation of harmful TMA and short-chain fatty acids. Novel investigations have focused on the inhibition of the conversion of TMA to the pro-atherogenic compound TMAO, which may show promising effects in terms of slowing the atherosclerotic process [54]. External intake of certain beneficial probiotics may have beneficial effects on the inflammatory aspects of atherosclerosis.

7. Future Perspectives

Atherosclerosis is a complex progressive vascular disease and is a major cause of disability, morbidity and mortality. As the mean age of the population

gets older, atherosclerosis will continue to be a major concern regarding the quality and duration of life in the next century. The demographic and clinical predictors of atherosclerosis are well-defined. Genetic predisposition, dyslipidemia, hypertension, diabetes mellitus, smoking, sedentary lifestyle and unhealthy nutrition are all well-known predictors of atherosclerosis. Moreover, numerous biochemical blood markers including hematological indices, homocysteine, uric acid and inflammatory markers have been proposed as contributors to atherosclerosis. Control of the clinical contributors, especially dyslipidemia and diabetes, is associated with more favorable clinical endpoints associated with atherosclerosis. However, treating the inflammatory aspects of atherosclerosis is still debatable. Infectious agents are the leading cause of the inflammatory response in the body and numerous infectious agents have been proposed to have an impact on the progression of atherosclerosis. *C. pneumoniae* was the first infectious agent isolated from atherosclerotic plaques. Antibiotic therapy to eradicate *C. pneumoniae* infection did not change the progression of atherosclerosis. These studies indicate the effect of multiple infectious agents in atherosclerosis. Recent studies have focused on the effect of oral flora and gut microbiota. The number of microbes in the gastro-intestinal flora is greater than the total number of eukaryotic cells in the body. Translocation of such bacteria to the blood stream and colonization of the vascular wall is easy; thus, simple tooth-brushing may cause transient bacteremia. Simple antibiotic use for a local infection may change the microbial diversity in the bowel and facilitate the colonization and translocation of virulent microbes. For a definitive explanation of the inflammatory aspects of atherosclerosis, future investigations concerning the oral and gut microbiota should clarify the linkage between infectious agent and atherosclerosis. We already know that certain strains in the oral and gut flora are associated with atherosclerosis. Colonization of relatively healthy strains may decrease the risk of atherosclerotic vascular disease. However, it is not known whether the inoculation of some strains has an additional beneficial effect on the progression of atherosclerosis. Thus, we can form a hypothesis similar to HDL cholesterol management. Lower HDL cholesterol levels are the strongest risk factor for atherosclerosis. Bringing up HDL levels to the normal range may attenuate the atherosclerotic process. However, the elevation of HDL over the normal range does not have a beneficial effect; on contrary, it might have deleterious effects in atherosclerosis. We can propose a similar scenario in the correlation between the gut microbiota and atherosclerosis. All the beneficial effects of the gut microbiota were defined in the absence of the deleterious effects of virulent microorganisms. So, we can propose that a beneficial gut microbiota is a relative term to describe the elimination of the negative impact of certain virulent strains. It means that we may define innocent microorganisms as beneficial, although they might have neutral effects on atherosclerosis. Colonization of the gut mucosa with a single bacterial species which was previously approved as beneficial may not show favorable effects on atherosclerosis and might even be harmful. To reach a more definitive conclusion, further investigations should be perform to show a correlation between probiotics that are assumed to be beneficial and slowing down the progression of atherosclerosis. The current unarguable truth is that keeping away some predefined strains from the gastro-intestinal microbiota may have favorable effects on atherosclerosis.

Definitive treatment of the inflammatory aspects of atherosclerosis may become more convincing after large-scale future investigations. Inhibition of IL-1β is a promising therapy and may become commercially available for clinical usage. However, the debate over specific therapies for microbial agents and atherosclerotic progression will continue.

8. Executive Summary

8.1. Atherosclerosis

Atherosclerosis is chronic, progressive inflammatory disease of the arterial wall. It is a leading cause of morbidity and mortality all over the world. The clinical and demographic contributors of atherosclerosis are well-defined. Systemic diseases including dyslipidemia, diabetes, hypertension and renal failure are the major proposed predisposing factors for atherosclerosis. Genetic predisposition, smoking, unhealthy lifestyle are the other basic factors implicated in the development of atherosclerosis.

8.2. Inflammation and atherosclerosis

The general consensus states that inflammation plays a central role in the development of atherosclerosis. The inflammatory cascade in the vascular wall that initiates the atherosclerotic process is well-defined. Direct analysis of atherosclerotic plaques has shown prominent infiltration of inflammatory cells and cytokines. In addition, serum

inflammatory markers, including hematological indices, CRP, IL-1, IL-6, procalcitonin and some cytokines, have been reported as predictors of atherosclerosis. However, the predictors and contributors of the inflammatory response in the vascular wall are still an arguable issue. Systemic and local inflammation, which enhance the burden of inflammation in the vascular wall, have been proposed as risk factors for the progression of atherosclerosis. Moreover, some systemic autoimmune diseases like rheumatoid arthritis and psoriasis may enhance inflammation in the vascular wall.

8.3. Microbial agents and atherosclerosis

Infectious micro-organisms are one of the major triggering factors of local and systemic inflammation. Microbial agents may accentuate inflammation in the vascular wall by increasing the intensity of the systemic inflammatory response. To clarify the inflammatory aspects of atherosclerosis, numerous investigations have been performed to show the linkage between microbial agents and atherosclerosis.

8.4. Chlamydia pneumoniae and atherosclerosis

Chlamydia pneumoniae was the first microbial agent isolated from atherosclerotic plaques. Analysis of both carotid and coronary atherosclerotic plaques showed the dissemination and colonization of the *C.pneumoniae* within the vascular wall and in atherosclerotic plaques. It was also reported that carotid plaques infected with *C. pneumonia*e tend to complicate and lead to clinical syndromes more frequently. Subsequently, additional bacterial and viral agents have been isolated from human atherosclerotic plaques.

8.5. Microbiota and atherosclerosis

After proposing a linkage between atherosclerosis and microbial agents, further investigations were focused on the impact of the oral and gut flora. Translocation and bacteremia with the oral and gut microbiota have been observed. Simple toothbrushing may lead to transient bacteremia. Antibiotics and an unhealthy diet may easily change the composition of the gut microbiota. The colonization of certain bacterial species may enhance atherosclerosis, either directly or indirectly. The accumulation of trimethylamine by certain detrimental bacterial species may accelerate the atherosclerotic process.

8.6. Conclusion and future perspectives

These studies concluded that micro-organisms have an important effect on the initiation, progression and complication of atherosclerotic plaques. Although initial antibiotic treatment trials failed to show an effect on atherosclerosis, recent investigations with gut microbiota have shown more promising results. The prevention of colonization with certain strains in the gut flora may have beneficial effects on the progression of atherosclerosis. Further investigations would clarify the cause and effect linkage between microbiological agents and atherosclerosis.

Author contribution

Conception and design of Study, Literature review, Acquisition of data, Analysis and interpretation of data, Research investigation and analysis, Data collection, Drafting of manuscript, Revising and editing the manuscript critically for important intellectual contents, Data preparation and presentation, Supervision of the research, Research coordination and management, Funding for the research: Ahmet Karabulut.

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