The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Review

J Atheroscler Thromb, 2024; 31: 189-200. http://doi.org/10.5551/jat.RV22015

Cigarette Smoking and Atherosclerotic Cardiovascular Disease

Mari Ishida[1], Chiemi Sakai[1], Yusuke Kobayashi[2] and Takafumi Ishida[3]

[1]Department of Cardiovascular Physiology and Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.
[2]Department of Cardiovascular Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.
[3]Department of Cardiovascular Medicine, Fukushima Medical University, Fukushima, Japan.

The detrimental effects of cigarette smoking on cardiovascular health, particularly atherosclerosis and thrombosis, are well established, and more detailed mechanisms continue to emerge. As the fundamental pathophysiology of the adverse effects of smoking, endothelial dysfunction, inflammation, and thrombosis are considered to be particularly important. Cigarette smoke induces endothelial dysfunction, leading to impaired vascular dilation and hemostasis regulation. Factors contributing to endothelial dysfunction include reduced bioavailability of nitric oxide, increased levels of superoxide anion, and endothelin release. Chronic inflammation of the vascular wall is a central pathogenesis of smoking-induced atherosclerosis. Smoking systemically elevates inflammatory markers and induces the expression of adhesion molecules and cytokines in various tissues. Pattern recognition receptors and damage-associated molecular patterns play crucial roles in the mechanism underlying smoking-induced inflammation. Smoking-induced DNA damage and activation of innate immunity, such as the NLRP3 inflammasome, cyclic GMP–AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, and Toll-like receptor 9, are shown to amplify inflammatory cytokine expression. Cigarette smoke-induced oxidative stress and inflammation influence platelet adhesion, aggregation, and coagulation via adhesion molecule upregulation. Furthermore, it affects the coagulation cascade and fibrinolysis balance, causing thrombus formation. Matrix metalloproteinases contribute to plaque vulnerability and atherothrombotic events. The impact of smoking on inflammatory cells and adhesion molecules further intensifies the risk of atherothrombosis. Collectively, exposure to cigarette smoke exerts profound effects on endothelial function, inflammation, and thrombosis, contributing to the development and progression of atherosclerosis and atherothrombotic cardiovascular diseases. Understanding these intricate mechanisms highlights the urgent need for smoking cessation to protect cardiovascular health. This comprehensive review investigates the multifaceted mechanisms through which smoking contributes to these life-threatening conditions.

Key words: **Smoking, Inflammation, DAMPs, Endothelial dysfunction, Atherothrombosis**

1. Introduction

Cardiac and cerebrovascular diseases are the second and fourth leading causes of death in Japan, following malignant neoplasms, and together account for 21.6% (340,000 people a year) of all deaths worldwide[1)]. In Western societies, atherosclerosis and associated cardiovascular disease remain as the most frequent causes of death and morbidity, and more than 10% of cardiovascular deaths are thought to be attributed to cigarette smoking[2)]. Many epidemiological studies have been conducted on the association between smoking and atherosclerosis, and it is clear that smoking increases the incidence and mortality of diseases, including ischemic heart disease and cerebrovascular disease that are based on atherosclerosis[3, 4)]. The mechanisms underlying atherosclerosis, arterial thrombosis, and their clinical manifestations as cardiovascular diseases induced by cigarette smoking have been extensively studied but

Address for correspondence: Mari Ishida, Department of Cardiovascular Physiology and Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan E-mail: mari@hiroshima-u.ac.jp

Received: October 31, 2023 Accepted for publication: December 1, 2023

are still only partially understood. Cigarette smoke is an extremely complex aerosol that contains over 4,000 compounds, including nicotine, carbon monoxide, reactive oxygen species (ROS), reactive nitrogen species, nitric oxide (NO), polycyclic hydrocarbons, acrolein, and other metals and oxidants[5]. Many studies have suggested that nicotine, carbon monoxide, and ROS are responsible for the pathogenesis and progression of smoking-induced atherosclerosis[6, 7]. The oxidant compounds in cigarette smoke induce oxidative stress on the endothelium, which results in endothelial dysfunction and injury, leading to the initiation of the atherosclerotic process. Importantly, however, a single constituent or a group of compounds in cigarette smoke is not likely to be the one associated with endothelial dysfunction[8] or atherosclerosis, but a very complex and variable mixture of constituents are involved in atherosclerosis initiation and progression as well as cardiovascular outcomes[9].

Many literatures have strongly suggested that cigarette smoke exerts an adverse effect on all stages of atherosclerosis, not just atherogenesis. The major pathogenesis of atherosclerosis induced by cigarette smoke is chronic inflammation of the vascular wall. Recent studies have suggested that damage-associated molecular pattern (DAMPs) is involved in the inflammation leading to atherosclerosis[10]. DAMPs are molecular structures released from damaged cells and tissues as well as necrotic cells and are involved in the induction of innate immunity and inflammation[11]. Ferroptosis is also induced by cigarette smoke[12] and may contribute to atherogenesis[13]. Cigarette smoke promotes platelet activation and facilitates platelet aggregation and adhesion to the site of endothelial injury[6]. Furthermore, it activates matrix metalloproteinases (MMP), thereby promoting vulnerable plaque formation and rupture[6]. These multifaceted processes highlight the detrimental impact of cigarette smoking on endothelial function, inflammation, and thrombosis, showing the importance of smoking cessation for cardiovascular health.

This review highlights the key process involved in the formation and progression of atherosclerosis caused by smoking, including endothelial dysfunction and damage, induction of inflammation, and shift toward a procoagulant state in the atherosclerotic regions and circulation, while introducing recent research in this field.

2. Endothelial Dysfunction

The vascular endothelium produces important vasodilators with anti-atherosclerotic and anti-aggregatory effects, such as NO and prostacyclin, and thus is a highly dynamic organ possessing anti-inflammatory, antithrombotic, and vasodilatory properties. Exposure to cigarette smoke leads to endothelial dysfunction, which is considered as the earliest manifestation of atherosclerosis, resulting in impaired regulation and maintenance of vascular dilation and hemostasis[14]. Endothelial function in humans is often measured via flow-mediated dilatation (FMD) in the forearm. Celermajer *et al.* published a study demonstrating that continuous smoking dose-dependently lowers FMD in healthy young adults[15]. This smoking-induced reduction in endothelium-dependent vasodilatation is reversible, with significant improvements in FMD reported after 1 year of smoking cessation[16]. A similarly strong association between passive smoking and FMD was reported, which was also demonstrated to be reversible 1 year after exposure cessation[17].

Numerous clinical and *in vitro* studies have demonstrated that endothelial dysfunction induced by cigarette smoke is mediated by a lack of NO bioavailability, production of superoxide anions, and increased production and release of endothelin[18] **(Fig. 1)**. ROS formation and dysfunctional endothelial NO synthase (eNOS) have been reported to contribute to the etiology of smoking-induced atherosclerosis[19]. Heitzer *et al.* reported that chronic smoking induces endothelial dysfunction and that antioxidant vitamin C restores endothelial dysfunction, suggesting that ROS are involved in the reduced vascular NO availability in endothelial dysfunction. In addition to the large amounts of free radicals and pro-oxidants such as NO, NO_2, peroxynitrite, phenol, and nitrosamines in the vapor phase of cigarette smoke, the particle phase contains high concentrations of quinones, which in biological systems undergo redox cycles to further produce O_2^- and H_2O_2 and other oxidant species. O_2^- in cigarette smoke is transported through the bloodstream to the vascular endothelium, where it reacts with NO to induce the formation of the highly cytotoxic peroxynitrite anion ($ONOO^-$). Together, these have been suggested to reduce vasoactive levels of NO and decrease response to endothelium-dependent vasodilators. Furthermore, tetrahydrobiopterin (BH4), a cofactor for eNOS, was associated with improved vasodilation to acetylcholine in chronic smokers, but not tetrahydroneopterin (NH4), suggesting that reduced NO bioactivity in chronic smokers is partially caused by reduced BH4 availability and that NOS-mediated superoxide production is an important source of superoxide in chronic smokers[20]. Barua *et*

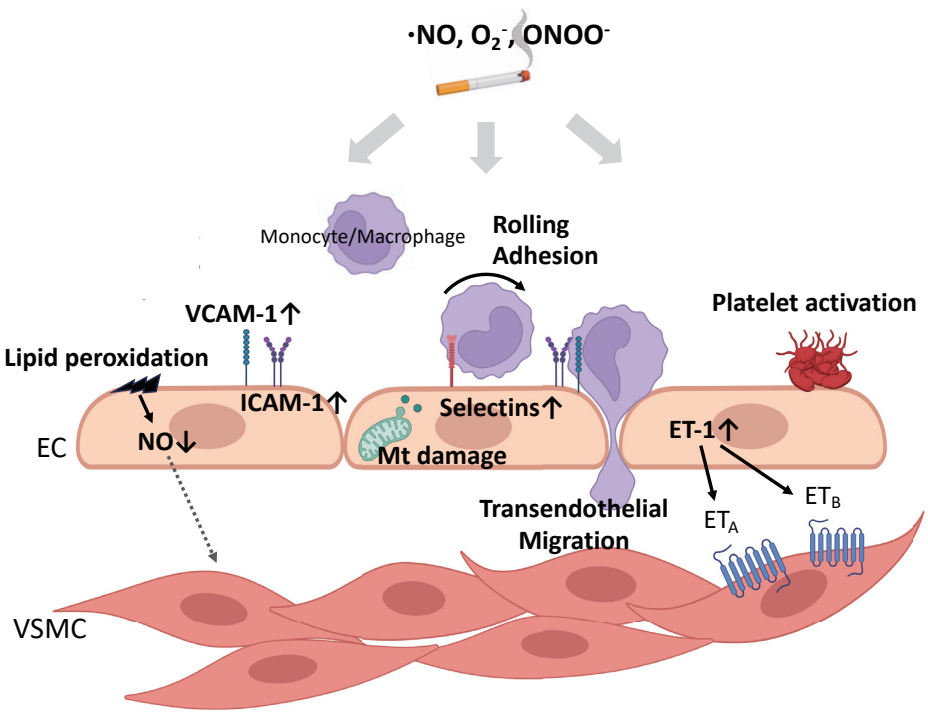

Fig. 1. Cigarette smoke-induced endothelial dysfunction

Free radicals and peroxynitrite anion ($ONOO^-$) from the vapor phase of cigarette smoke or produced via redox cycles activate ECs by inducing endothelial selectins (P- and E-selectin), ICAM-1, VCAM-1, and platelets. Monocyte adhesion and transendothelial migration are induced by increased adhesion molecule expression. Endothelial activation is characterized by reduced NO availability and results in the loss of function of vascular smooth muscle cells. Smoking-induced endothelial dysfunction is associated with increased ET-1 release. Smoke extract disrupts mitochondrial function and can trigger inflammation via damage-associated molecular patterns. Inflammatory and proatherogenic cytokines released from EC in response to smoke exposure further lead to endothelial dysfunction (refer to the section on inflammation). EC, endothelial cells; VSMC, vascular smooth muscle cells; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NO, nitric oxide; ET-1, endothelin 1. Created using Biorender.com.

al. reported that vascular endothelial cells cultured in a culture medium supplemented with smoker's serum exhibited decreased NO production, decreased eNOS activity, and increased endogenous ROS production compared with nonsmokers, which were restored by antioxidant drugs[21]. Activation of xanthine oxidase[22], nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)[23], and mitochondrial respiratory chain[24] have also been reported to contribute to ROS increase in endothelial cells exposed to cigarette smoke. In addition, it was found that cigarette smoke extract (CSE), a water-soluble tobacco smoke component, induces a moderate level of mitochondrial outer membrane permeabilization (MOMP) that does not trigger apoptosis, namely, minority MOMP, while also diminishing the mitochondrial membrane potential in endothelial cells. This induction results in the leakage of mitochondrial contents, including electrons, mitochondrial DNA, and cytochrome C, into the cytosol[25]. A decrease in mitochondrial membrane potential and resultant mitochondrial dysfunction leads to an increase in ROS[26]. This phenomenon also contributes to the development of inflammation through DAMPs, which will be discussed in the next section.

3. Inflammation

As the major pathogenesis of atherosclerosis, chronic inflammation of the vascular wall plays a significant role in its development and progression[9]. Although cigarette smoking is widely known to induce inflammation, the detailed mechanisms by which smoking habits induce inflammation in the vascular wall are still under investigation. Smoking has been clinically demonstrated to systemically and locally impact and activate the immune system. Among smokers, an increase in neutrophils, lymphocytes, and monocytes compared with nonsmokers have been reported[27]. Smokers also exhibit significantly elevated serum levels of inflammatory cytokines such as tumor necrosis factor-α and interleukin (IL)-1β[28]. As

mentioned later, experiments conducted *in vitro* have demonstrated that CSE increases the expression of IL-6 and IL-8 [12, 25]. However, to the best of our knowledge, no consistent reports indicating that smoking elevates IL-6 and IL-8 concentrations in human blood have been conducted. However, there are reports suggesting that smoking may not alter the blood concentrations of IL-6 and IL-8 [29] and others indicating an increase in IL-6 and IL-8 concentrations in the saliva of smokers[30]. Smoking and secondhand smoking have also been shown to increase serum levels of the inflammatory marker C-reactive protein (CRP)[31].

In vitro experiments have reported various aspects of cigarette smoke-induced inflammation. Cigarette smoke has been demonstrated to induce the expression of adhesion molecules on endothelial cell surfaces[32] and the release of atherosclerosis-promoting cytokines such as IL-6 and IL-8 [25, 32]. We demonstrated that NF-κB activation is involved in the upstream regulation of IL-6 in the endothelial cells[25]. Additionally, in human lung epithelial cells and fibroblasts, tobacco smoke activates mitogen- and stress-activated kinase, leading to the activation of NF-κB-dependent inflammatory genes, including IL-6, IL-8, and COX-2, accompanied by changes in chromatin structure[33].

Vascular inflammation is multifactorial. Although the innate immune system is essentially responsible for host defense against foreign organisms, accumulating evidence indicates the involvement of the innate immune system in vascular inflammation[34, 35]. Recent insights also proposed that dysregulation of innate immunity triggers chronic inflammation across diverse organs, culminating under conditions such as atherosclerosis, autoimmune diseases, malignancies, and age-related deseases[36]. Pattern recognition receptors (PRRs) play a critical role in the proper functioning of the innate immune system[37], directly recognizing specific molecular structures, such as pathogen-associated molecular patterns (PAMPs), from microbial pathogens and DAMPs released from host cells upon damage or death. Various cells present in atherosclerotic lesions, including endothelial cells, macrophages, and dendritic cells, express Toll-like receptors (TLRs), a type of PRRs[38]. The most extensively researched in the context of atherosclerosis is TLR9, which recognizes unmethylated CpG (cytosine–phosphate–guanine dideoxynucleotide) DNA, derived from bacteria and viruses[39], as well as self-derived DNA[40]. This recognition activates TLR9 and initiates various innate immune responses. In mouse experiments, TLR9 has been demonstrated to play a pivotal role in the development of atherosclerosis. For example, inhibition of TLR9 activation using immune-regulatory oligodeoxynucleotides transforms macrophage plasticity into anti-inflammatory macrophages and suppresses atherosclerotic plaque formation[41]. Conversely, TLR9 agonist administration through intravenous injection promotes plaque formation in apolipoprotein E (ApoE)-deficient mice[42]. As regards the impact of smoking, exposure to e-cigarette vapor has been reported in ApoE-deficient mice to increase TLR9 expression in monocytes, leading to accelerated atherosclerotic lesions[43] **(Fig. 2)**. TLR9 antagonist administration before e-cigarette vapor exposure not only mitigated TLR9 upregulation in atherosclerotic plaques but also suppressed the increase in plasma levels of inflammatory cytokines and accumulation of lipids and macrophages in the plaques. Furthermore, cell-free mitochondrial DNA isolated from macrophages treated with e-cigarette vapor extract was demonstrated to activate TLR9 in reporter cells, and TLR9 inhibition suppressed the induction of inflammatory cytokines in macrophages[43].

The involvement of inflammasome in atherosclerosis progression has been reported[44]. NLRP3 is an intracellular sensor that detects a broad range of PAMPs as well as self- and foreign-derived DAMPs, such as ATP, cholesterol crystals, oxidized mitochondrial DNA, cyclic GMP–AMP, and nanoparticle, resulting in the formation and activation of the NLRP3 inflammasome[45]. AIM2 is an inflammasome component distinct from NLRP3 and functions as a DNA sensor protein that recognizes bacterial and viral DNA, as well as endogenous double-strand DNA[46]. Inflammasome assembly leads to caspase 1-dependent release of proinflammatory cytokines IL-1β and IL-18 as well as to gasdermin D (GSDMD)-mediated pyroptotic cell death, facilitating the extracellular release of IL-1β and IL-18 [45]. Transplantation of bone marrow cells lacking NLRP3, ASC, and IL-1α/β into atherosclerosis-prone low-density lipoprotein receptor (LDLR)-deficient mice reduced the size of atherosclerotic lesion[47]. Consistently, ApoE-deficient mice lacking caspase-1/11 and LDLR-deficient mice lacking caspase-1/11 exhibited reduced atherosclerosis progression[48, 49]. These findings provide direct evidence of the contribution of the NLRP3 inflammasome to the progression of atherosclerosis. Furthermore, increased AIM2 expression and accumulation of extracellular double-strand DNA were observed in ApoE-deficient mice with advanced atherosclerosis[50]. Overexpression of AIM2 in ApoE-deficient mice fed a high-fat diet resulted in the formation of plaques with more pyroptotic vascular

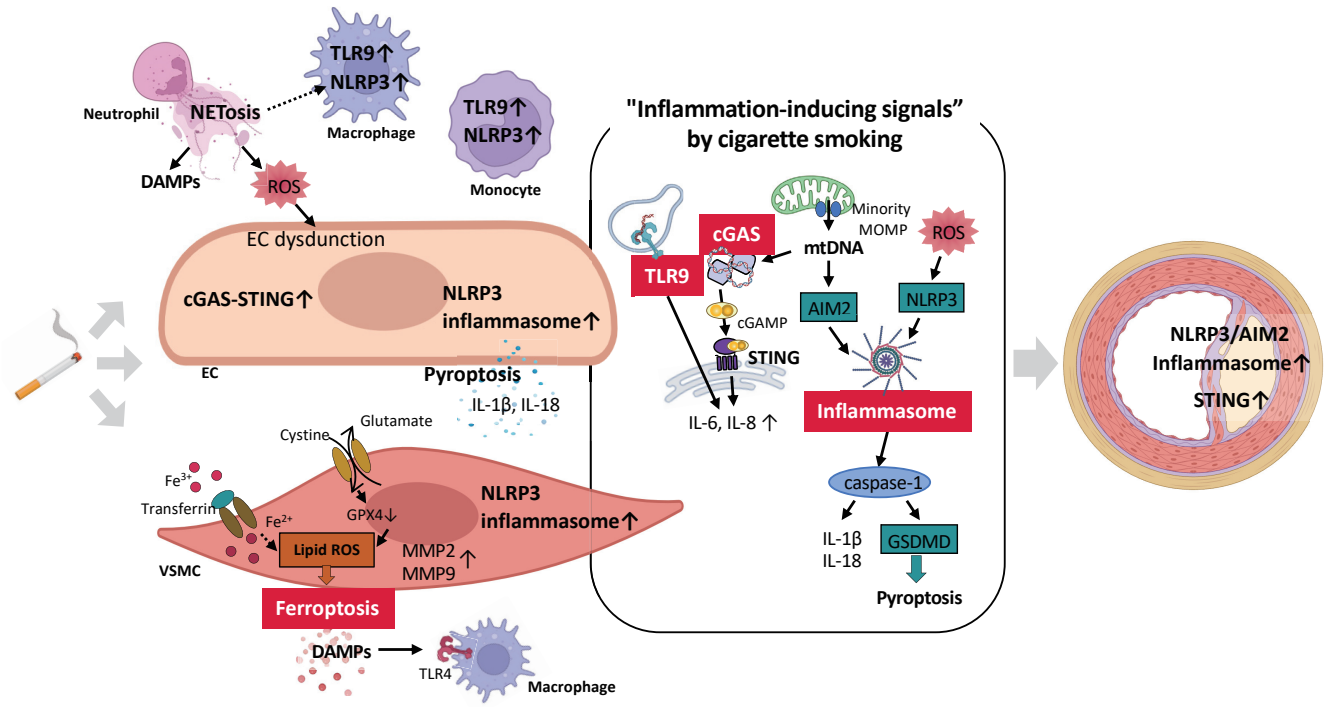

Fig. 2. Schematic diagram of the mechanisms of inflammation induced by smoking

The pathogenesis of atherosclerosis is mainly driven by chronic inflammation in the vascular wall, with cigarette smoking as an inflammatory trigger. The innate immune system, involving pattern recognition receptors, plays a pivotal role, particularly TLR9, NLRP3/AIM2 inflammasome, and cGAS-STING, in atherosclerotic lesions. Cigarette smoke-induced cGAS is mainly activated by cytosolic-free mtDNA released through minority MOMP. Both the TLR9 and cGAS pathways lead to an increased expression of inflammatory cytokines, such as IL-6 and IL-8. Cytosolic-free mtDNA also activates DNA-sensing AIM2 inflammasome, and cigarette smoke-induced ROS activates NLRP3 inflammasome; both induce GSDMD-mediated pyroptosis and release of proinflammatory cytokines such as IL-1β and IL-18 into the extracellular space. In addition, cigarette smoke triggers another form of cell death, ferroptosis, leading to the activation of redox-sensitive transcription factor, MMP expression, and inflammation in VSMC. Damaged tissues release DAMPs, contributing to innate immunity and inflammation. Other mechanisms such as NETs generate ROS and DAMPs and activates NLRP3 inflammasome in macrophages; they also contribute to atherogenesis. The cell-specific mechanisms of inflammation induced by smoking is presented in this figure. EC, endothelial cell; VSMC, vascular smooth muscle cell; TLR, Toll-like receptor; cGAS, cyclic GMP–AMP synthase; STING, stimulator of interferon genes; mtDNA, mitochondrial DNA; MOMP, mitochondrial outer membrane permeability; IL, interleukin; ROS, reactive oxygen species; GSDMD, gasdermin D; MMP, matrix metalloprotease; DAMPs, damage-associated molecular patterns; NETs: neutrophil extracellular traps. Created using Biorender.com.

smooth muscle cells and increased macrophage recruitment within the plaques[51]. These studies collectively highlight the pivotal role of inflammasome in atherosclerosis progression. In the context of atherosclerosis development caused by cigarette smoke, NLRP3 inflammasome activation has been reported to be crucial (**Fig. 2**). Exposure to cigarette smoke has been shown to activate NLRP3 inflammasome in various cell stages, including monocytes, macrophages, and foam cells, contributing to the initiation, progression, and advancement of atherosclerosis[52]. Interestingly, cigarette smoke condensate induced differentiation of THP-1 monocytes into macrophages, resulting in stage-specific activation of the NLRP3 inflammasome and increased IL-1β and IL-18 secretion[52]. Furthermore, nicotine treatment in rat vascular smooth muscle cells and rat aortic tissues resulted in increased ROS production, NLRP3 inflammasome activation, and CRP elevation[53]. In ApoE-deficient mice fed a high-fat diet, nicotine amplified atherosclerotic lesions and increased inflammatory cytokines, possibly through ROS-NLRP3-mediated endothelial cell pyroptosis[54], as nicotine activated NLRP3 through ROS, leading to pyroptosis as well as IL-1β and IL-18 production in human aortic endothelial cells[54]. Clinical research has reported increased transcriptional and translational expressions of NLRP3 inflammasome markers (caspase-1, pro-IL-1β, IL-1β, pro-IL-18, and IL-18) in the monocytes of smokers with coronary artery disease (CAD) compared with nonsmokers with CAD[55]. In addition, oxidative stress markers (8-isoprostane and 8-oxo-2′-deoxyguanosine), caspase-1, IL-1β, and IL-18 in serum were found to

be elevated in smokers with CAD[55].

Cyclic GMP–AMP synthase (cGAS), known as a cytosolic DNA sensor, is activated not only by pathogen-derived DNA but also by endogenous DNA. It is expressed in various cells, including microglia, neurons, hepatocytes, peripheral monocytes, dendritic cells, macrophages, and cardiovascular cells, such as endothelial cells, vascular smooth muscle cells, cardiomyocytes, and fibroblasts[56]. cGAS catalyzes the production of the second messenger 2'3'-cGAMP, which activates the endoplasmic reticulum-associated stimulator of interferon genes (STING), ultimately leading to the induction of type I interferons and inflammatory genes[56]. In animal models of atherosclerosis, the cGAS-STING pathway has been demonstrated to play a pivotal role in the induction of inflammation in atherosclerosis development. In ApoE-deficient mice fed a high-fat diet, DNA damage markers and STING protein expression were elevated in aortic macrophages, and pharmacological inhibition or genetic deletion of STING improved atherosclerosis in these mice[57]. These results suggest the involvement of STING in the progression of atherosclerosis induced by administration of a high-fat diet. We recently demonstrated that water-soluble CSE induces nuclear and mitochondrial DNA damage in vascular endothelial cells, leading to an increase in cytosolic DNA, a DAMP. This, in turn, activates the cGAS-STING pathway, leading to the upregulation of cGAS-STING-dependent inflammatory cytokine expression[25] **(Fig. 2)**. While both nuclear and mitochondrial DNA were increased by CSE, mitochondrial DNA has been identified as a main contributor to IL-6 induction among inflammatory cytokines[8]. Smokers also exhibit increased levels of circulating cell-free DNA derived from both nuclear and mitochondrial sources, indicating the potential to further exacerbate systemic inflammation[25]. Collectively, these findings highlight the pivotal role of PRR- and DNA sensor-mediated signaling through DAMP recognition in cigarette smoke-induced inflammation.

As aforementioned, DAMPs are molecular structures released from damaged tissues or necrotic cells, contributing to the induction of innate immunity and inflammation. Various forms of cell death have been suggested to be triggered by cigarette smoke, leading to the underlying cause of atherosclerosis[58]. Ferroptosis, a recently identified cell death, has been reported to be induced by CSE in vascular smooth muscle cells, distinct from apoptosis or necrosis[12] **(Fig. 2)**. The central feature of ferroptosis is the presence of redox-active iron and dysfunction of lipid peroxidation[59]. Accumulating evidence suggests that ferroptosis significantly influences the regulation of oxidative stress and inflammatory responses and is implicated in atherosclerotic cardiovascular diseases[59]. Sampilvanjil *et al.* reported that CSE-induced increases in the expression of inflammatory cytokines, such as IL-1β and IL-6, as well as MMP-2 and MMP-9 in vascular smooth muscle cells, are inhibited by the ferroptosis inhibitor ferrostatin-1[12]. Furthermore, *ex vivo* experiments revealed that CSE induces medial loss in aortic tissue, which is suppressed by ferrostatin-1. These findings indicate that CSE-induced ferroptosis releases DAMPs such as nucleic acids and high mobility group box 1, recognized by innate immune receptors such as TLR4 on macrophages[60], ultimately enhancing inflammation **(Fig. 2)**. The fact that the mechanism of ferrostatin-1 involves radical scavenging of membrane lipids supports the possibility that lipid peroxidation regulates redox-sensitive transcription factors such as NF-κB and AP-1, which play pivotal roles in the expression of cytokines and MMPs. These insights imply the involvement of vascular smooth muscle cell loss, inflammation, and matrix degradation induced by CSE in the development of aortic aneurysms and dissections, for which smoking is a major risk factor.

Other molecular mechanisms such as neutrophil extracellular traps (NETs) and autophagy produce DAMPs and modulate innate immunity. Their role in the pathogenesis of atherosclerosis is also currently investigated. NETs are structures composed of chromatin containing released DNA that are formed during neutrophil activation. These structures are dispersed with various proteins and play a crucial role in the capture and sterilization of microorganisms in the extracellular environment. Recent evidence suggests that NETs are implicated under various pathophysiological conditions, including atherosclerosis, thrombosis, autoimmune diseases, cancer, diabetes, and Alzheimer's disease. NETs have been observed in atherosclerotic lesions of both human and animal models, and their involvement in diverse mechanisms leading to atherogenesis has been reported[61, 62]. In particular, NETs induce oxidative stress, promote endothelial cell dysfunction and apoptosis, and contribute to the development of inflammation by triggering the generation of DAMPs, as aforementioned. In addition, they form a fibrin-like matrix for platelet adhesion, activation, and aggregation. They also promote the accumulation of thrombosis-promoting molecules such as von Willebrand factor and fibrinogen, significantly contributing to thrombus formation and propagation. Nicotine has been reported to induce NETs in

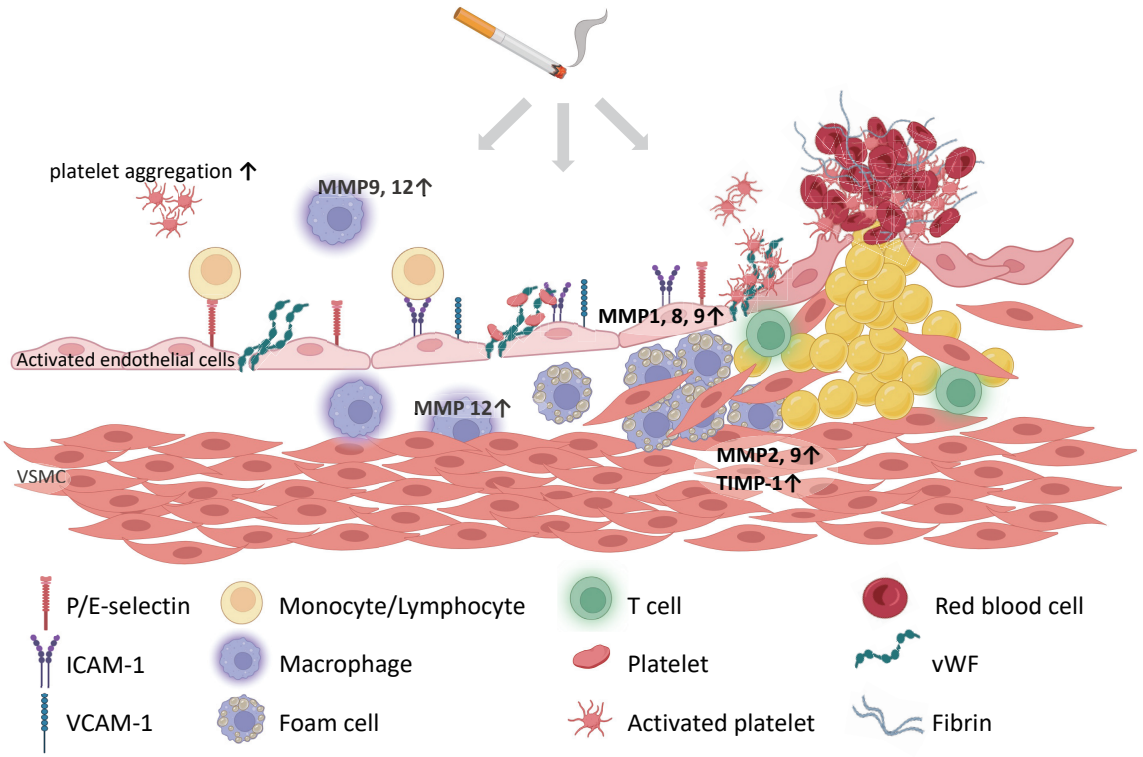

Fig. 3. Cigarette smoke-induced atherothrombosis

Cigarette smoke activates endothelial cells, which enhances the expression of endothelial selectins (P- and E-selectin), ICAM-1, and VCAM-1, promoting the adhesion of monocytes/lymphocytes and platelets to inflammatory endothelium. Expression of vWF expressed in the exposed subendothelial tissue from injured dysfunctional endothelial cells promotes platelet adhesion and aggregation reactions, leading to platelet thrombus formation. Moreover, smoking contributes to the formation of vulnerable plaques through increased inflammation and upregulated activity of MMPs. Exposure to cigarette smoke induces the expression of MMP-9 and MMP-12 in macrophage, MMP-1, MMP-8, and MMP-9 in the endothelial cells, and MMP-2 and MMP-9 in VSMC. ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor; MMP, matrix metalloprotease; VSMC, vascular smooth muscle cells. Created using Biorender.com.

neutrophil-like cells derived from HL-60 cells[63] (Fig. 2). NETs induced by nicotine negatively regulate autophagy in macrophages, resulting in the activation of inflammasome[63]. Inhibition of NET formation by depleting neutrophil peptidylarginine deiminase 4 in neutrophil leukocytes results in reduced atherosclerotic plaques in ApoE-deficient mice treated with nicotine. This suggests that NETs promote atherogenesis via inflammasome activation by inhibiting macrophage autophagy[63].

4. Thrombotic Effects

Among patients with acute coronary syndrome, smokers have a higher incidence of in-stent thrombosis than nonsmokers[64], and it has been reported that 75% of cases of sudden cardiac death due to acute atherothrombosis were smokers[65]. For quite some time, increased tendency for platelet aggregation and blood clotting in individuals exposed to cigarette smoke has been reported[66, 67] (Fig. 3). A positive association was demonstrated between the degree of smoking and the extent of platelet activation both *in vivo*[68] and *in vitro*[69]. Multiple mechanisms for the increased platelet aggregation in smokers have been suggested. An increased platelet count[70] and increased circulating levels of platelet-activating factor (PAF) and PAF-like lipids have been found in smokers[71]. Some studies have suggested that oxidative stress plays a pivotal role in smoking-induced platelet activation. N-acetylcysteine, an antioxidant, is shown to protect against cigarette smoke-induced impairment of platelet-mediated ADP hydrolysis[72]. A previous study showed that long-term smoking impairs the release of platelet-derived NO[73]. This suggests an imbalance in platelet regulation resulting in increased platelet aggregation as NO is a potent platelet inhibitor. Proteomic analysis with the platelets from smokers revealed increased expression of proinflammatory molecules, including the platelet

fibrinogen receptor glycoprotein IIb/IIIa, compared with nonsmokers[74]. Exposure to cigarette smoke enhances platelet activation due to an increase in thromboxane A_2 formation[75]; it also promotes platelet-dependent generation of thrombin, a highly potent platelet activator, potentially leading to a positive-feedback loop for platelet activation and coagulation[76].

As aforementioned, endothelial cells are damaged and activated by cigarette smoke, becoming the sites for inflammation and thrombotic tendencies. In endothelial cells activated by smoking, increased generation of von Willebrand factor and thrombomodulin has been reported[77] **(Fig. 3)**. Exposing the subendothelial matrix to flowing blood promotes platelet activation and aggregation through interactions between platelet surface glycoprotein receptors, subendothelial collagen, and immobilized von Willebrand factor. This process is considered the initial stage of pathological thrombus formation[78]. In addition to increased platelet aggregation and coagulation, exposure to cigarette smoke has been reported to be correlated with a state of impaired fibrinolysis[6].

Increased inflammation and upregulated MMP activity contribute to the formation of vulnerable plaques[79, 80]. Exposure to cigarette smoke induces the expression of various MMPs in different cell types[81] **(Fig. 3)**. Cigarette smoke or CSE has been demonstrated to induce the expression of MMP-12, tumor necrosis factor, and tissue factor in lung alveolar macrophages and lung tissue[82, 83]. Furthermore, plaques obtained from smokers undergoing carotid endarterectomy exhibited increased expression of macrophage-derived MMP-12 compared with nonsmokers. In human aortic and coronary artery endothelial cells exposed to cigarette smoke or CSE, the expression of MMP-1, MMP-8, and MMP-9 increased[84], and nicotine has been shown to promote MMP expression in smooth muscle cells[85]. Several mechanisms underlying MMP expression have been reported. CSE induced the expression of MMP-2, MMP-9, and tissue inhibitor of metalloproteinases-1 (TIMP-1) in vascular smooth muscle cells, which was reportedly terminated by ferroptosis-specific inhibitors[12]. Cigarette smoke constituents responsible for ferroptosis induction in vascular smooth muscle cells include acrolein and methyl vinyl ketone. Acrolein and cigarette smoke were also demonstrated to upregulate MMP-1 expression and downregulate TIMP-3 expression in rabbit aortic endothelial cells, which was attributed to the inhibition of the mammalian target of rapamycin–p70S6K pathway. In ApoE-deficient mice, acrolein activated MMP-9 in human macrophages and stimulated MMP activity in an oxidative stress-dependent manner in advanced atherosclerotic lesions[86]. The association between myocardial infarction and cardiovascular disease prevalence and exposure to acrolein-rich air pollution is well documented[87], indicating that MMP activation may play a critical role as an instability factor in acute plaques.

In general, systemic inflammation is associated with plaque rupture and the occurrence of acute coronary syndrome[88], highlighting the importance of inflammation in plaque vulnerability. Furthermore, cigarette smoke may induce the accumulation of circulating monocytes within the fibrous cap of stable plaques by promoting the expression of adhesion molecules[89] and monocyte chemoattractant protein-1 [90]. Upon plaque rupture, platelet adhesion and aggregation, coagulation cascade activation, and fibrinolysis balance are all affected via complex interactions. As aforementioned, cigarette smoke triggers inflammation in cells, leading to increased expression of inflammatory cytokines[91] and adhesion molecules[89]. Inflammatory endothelial cells enhance the expression of endothelial E- and P-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 **(Figs. 1 and 3)**, promoting the firm adhesion of platelets to inflammatory endothelium via interactions with platelet receptors, ultimately leading to the formation of occlusive intravascular thrombus[92, 93]. Furthermore, there exists a positive-feedback loop in which platelet-derived bioactive molecules such as IL-1 and CD40 ligand stimulate NF-κB-dependent upregulation of endothelial adhesion molecules, further facilitating adhesion of platelets and white blood cells to the arterial wall[94].

5. Conclusions and Future Perspective

Cigarette smoking has well-established detrimental effects on cardiovascular health, with a particular focus on atherosclerosis and atherothrombosis. This comprehensive review has highlighted the intricate mechanisms by which smoking contributes to such life-threatening conditions, including endothelial dysfunction, inflammation, and atherothrombotic effects. In summary, endothelial dysfunction, characterized by reduced NO bioavailability and increased superoxide anion production, is one of the earliest manifestations of smoking-induced atherosclerosis. Smoking-induced inflammation is a central pathogenic factor in smoking-induced atherosclerosis, with some involvement in inflammatory response mediated by

PRRs and DAMPs. These mechanisms induce chronic vascular wall inflammation and contribute to atherosclerotic progression. Furthermore, cigarette smoke exerts a direct influence on atherothrombosis by enhancing platelet activation, adhesion, and aggregation as well as perturbing the coagulation cascade and fibrinolysis balance. MMPs further contribute to plaque vulnerability and atherothrombotic events. Endothelial dysfunction, inflammation, and atherothrombosis are correlated and mutually exacerbate each other. Considering the significant public health burden posed by cardiovascular diseases, particularly in smoking populations, it is necessary to highlight the urgent need for smoking cessation. Understanding these multifaceted mechanisms at the intersection of endothelial function, inflammation, and thrombosis emphasizes the importance of anti-smoking initiatives and therapies for the preservation of cardiovascular health. Future research should continue to explore the specific molecular pathways and potential therapeutic interventions targeting these mechanisms, ultimately providing more effective strategies for mitigating cardiovascular risks associated with cigarette smoking.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research (KAKENHI #21K080290 to M.I., KAKENHI #22K160760 to C.S., and KAKENHI#23K07557 to T.I.); a research grant from the Tsuchiya Memorial Medical Foundation to M.I.; a grant from the Smoking Research Foundation (M.I. and C.S.).

Competing Interests

T.I. is a chair of an endowed course by BIOTRONIK Japan, Inc., Abbot Medical Japan LLC, and Japan Lifeline Co., Ltd. Other authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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