Review Article

The Role of Inflammatory Agents in Endothelial Function and their Contribution to Atherosclerosis

EMMANOUIL N. KARATZIS

First Cardiology Department, Red Cross Hospital, Athens, Greece

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Address:
Emmanouil N. Karatzis

38 Sofokleous St., Glyfada 166 74 Athens, Greece
e-mail:
manoskaratzis@yahoo.com

In contrast to the older view that saw the endothelium as a passive barrier, recent bibliographical data portray it as perhaps the largest endocrine gland, with dynamic behavioural features including the continuous regulation of vascular tone.[1] This involves communication between cells of the vascular layers (endothelial, smooth muscle) and cells in the blood circulation (monocytes, lymphocytes, red blood cells, platelets). The continuing interaction creates a fragile dynamic balance.

In recent years, the inflammatory cascade that takes place on vascular endothelium has been established as the precursor process that, via a series of pathogenetic mechanisms, leads initially to vascular atheromatosis and subsequently to thrombotic episodes that affect both central and peripheral vessels.[2,3] It is generally believed that the inflammatory process can be triggered by any kind of endothelial "injury".

The serious consequences in terms of cardiovascular morbidity and mortality render it vitally important to elucidate the interacting mechanisms. The aim of this review is to provide a brief presentation of our current knowledge concerning inflammatory processes in the endothelium during atheromatosis.

Main events occurring in the inflammatory atheromatosis process and factors involved

In recent years, research efforts have revealed the existence of potent mediators of an inflammatory sequence that takes place in the vascular wall. These molecules are called proinflammatory cytokines and their action has been found to be either direct (autocrine), through special receptors in the surface of cells, or indirect (paracrine), by stimulating the production of other growth transmitters. They act on each other either synergistically or antagonistically.[4]

The presence of classical risk factors, such as oxidised lipoproteins, smoke, glycosylation products in diabetes mellitus, infections by Chlamydia or viruses, and elevated shear stress, can stimulate the production of cytokines by vascular endothelium. This is then the trigger for the atherogenic process, which is comprised of the following stages:[5]

1. The initial stage of the inflammatory process, which concerns dysfunctional vascular endothelium and the activation-migration of monocytes-macrophages.[5-7]

2. A further stage that involves the activation and proliferation of smooth muscle cells.

Of fundamental importance in atheromatosis and thrombotic complications is nuclear factor NF-kB.[7] This transcription factor activates a number of genes that are involved in the acute inflammatory reaction and play a crucial role. Its binding with I-kB in the cytoplasm of many cells (T-lymphocytes, monocytes, macrophages, endothelial and smooth muscle fibres) renders it inactive. However, a large number of lipopoly-

saccharides (LPS), interleukin(IL)-1 and tumour necrosis factor a (TNF-a) modify this binding and enhance its activation. Subsequently, NF-kB activates genes and increases the expression of almost all the factors involved in the inflammatory reaction (Table 1), and in this way it sets off the inflammation cascade. NF-kB activation is an event that has been observed to occur in the presence of most risk factors.[3,5,7] In addition, NF-kB regulates the expression of genes that code for its own synthesis (autoregulation mechanisms) and has been implicated in the process of atheromatous plaque rupture.

Another of the most classical indexes of inflammation is C-reactive protein (CRP), which is produced by the liver in response to both inflammatory and non-inflammatory stimuli. It participates in the activation of monocyte and complement recruitment, resulting in the secretion of inflammatory cytokines (Table 1) which have a feedback effect on its own secretion. The measurement and monitoring of CRP during a course of treatment are already widely used in clinical practice.

In 1987 Arfors et al[8] demonstrated that the "gradual recruitment" of white blood cells was the primary event in the inflammatory atheromatous sequence. The corresponding stages in this procedure are as follows:

Capture and rolling of leucocytes

Red blood cells move through the vessel lumen with a higher velocity than do white blood cells. This movement occurs in such a way that the leucocytes are pressed towards the periphery of the lumen, with the result that they approach endothelial cells very closely.[9] This leads to their rapid and powerfully bonded capture by groups of adhesion molecules, which also results in their contact with the vascular wall.[10] The L and E forms of selectin in combination with the integrins (β2-β3 and α4) have been found to be involved in leucocyte capture.[11,12]

The above molecules, along with the participation of IL-8, monocyte chemoattractant protein-1 (MCP-1) and the normal T-leucocyte expression and secretion (RANTES) factor,[7] are involved in starting leucocytes rolling along the endothelium at a very slow speed, well below than that of the arterial blood in the vessel concerned.[13] The form of the leucocytes then changes from spherical to spindle-shaped, so that a greater part of their surface comes into contact with the vascular wall.

Slow rolling and activation

The process of rolling has been shown to be extremely fundamental in the "recruitment" of leucocytes to regions outside the lumen and for specific tasks. In studies where the above process was inhibited, the migration of leucocytes decreased dramatically.[11] In effect, during slow rolling leucocytes are gradually activated[14] and prepared for firmer adhesion.

Firm adhesion and migration

The chemokines that are synthesised in large numbers of inflammatory cells[15] on the activation of integrins[12] and the intracellular adhesion molecule (ICAM)-1[16] participate in firm adhesion when the rolling has already become very slow. At this stage the leucocytes appear to become even more spindle-shaped in order to ensure the greatest possible area of surface contact with endothelial cells.

Migration effectively represents a subsequent stage during which the leucocytes "crawl" beneath the endothelial layer and are extravascularised into extracellular space. A large number of mediators have been reported to affect the permeability of the endothelium, such as 5-hydroxytryptamine, histamine, bradykinine, prostaglandins, and nitric oxide, either alone or in combination with proinflammatory cytokines and growth factors.[3,17]

The differentiation of monocytes into macrophages and the subsequent absorption of lipids (especially of oxidised LDL), leads to the formation of foam cells (Figure 1). The fatty streaks that are found in dissection preparations of vessels from children (or even adolescents) are the first macroscopic findings of the atheromatous process and are due to foam cells.[3,18] Next comes the proliferation of smooth muscle cells under the action of growth factors, and the concentration of more inflammatory cells leads to the formation of atheromatous plaque,[2,18] which consists of a fatty nucleus with atherothrombotic properties and a thin fibrous cap that separates its contents from the vessel lumen and prevents their contact with platelets. Thinning and disintegration of the cap (caused by metalloproteinases, which are proteolytic enzymes produced by activated macrophages), may lead to rupture, followed by the attraction and activation of platelets, the beginning of thrombus formation and eventually the initiation of acute ischaemic events.[3]

Interaction between nitric oxide and inflammatory factors. The importance of shear stress and the production of free oxygen radicals

An important part of the inflammatory sequence, which is also related with the location of atherosclerotic lesions,[19] has to do with mechanical forces and in

Table 1. Summary of the main inflammatory and anti-inflammatory agents, their ways of activation and main effects.

Inflammatory agents	Secretion activated by	Main effects
IL-1β [32,16,52]	Endotoxins, NF-kB.	Via endothelial stimulation, increase in synthesis and release of CRP, ICAM-1. Stimulation of NF-kB activation.
IL-6 [32]	Endotoxins, NF-kB.	Promotes the expression of adhesion molecules (integrins) in leucocytes and endothelial cells. Increases the synthesis and release of CRP.
IL-8 [7,54]	NF-kB.	Takes part in the leucocyte rolling and migration phase, together with selectins and MCP-1.
CRP [7,12,53]	IL-1β, IL-6, by inflammatory and non-inflammatory stimuli.	Promotes the conscription of monocytes (via MCP-1) and takes part in their firm adhesion. Activation of the complement, with secretion of IL-1β, IL-6, TNF-a. Causes expression of adhesion molecules ICAM-1, VCAM-1.
TNF-a [7,12,16,32,52]	Endotoxins, CRP, NF-kB.	Takes part in leucocyte slow rolling and with CRP prepares the ground for their firm adhesion. Via endothelial stimulation, increases ICAM-1 production. Promotes NF-kB activation.
MCP-1 [7,12]	Oxidative stress, CRP, NF-kB, by inflamed cells.	Leucocyte rolling and migration in combination with IL-8. Promotes NF-kB activation.
NF-kB [54]	Oxidative stress, shear stress, IL-1β, TNF-a and other chemokines(MCP-1)	Increases secretion of factors such as IL-1β, IL-6, IL-8, interferon-γ, TNF-a, MCP-1, ICAM-1, VCAM-1.
Anti-inflammatory agents	**Activated by**	**Main effects**
IL-1ra [7,25]	Inflammatory cytokines and chemokines, as well as risk factors.	Secreted from the endothelium in response to inflammatory reaction, especially that caused by IL-1β.
TGF-β [7,25]	Inflammatory cytokines, chemokines, hyperglycaemia, lipoproteins, smoking, oxidative stress and generally the inflammation cascade.	Secreted for the moderation of the inflammatory reaction. Reduces the expression of E-selectin, ICAM-1, VCAM-1, MCP-1. Reduces TNF-a receptors and reverses endothelial dysfunction.
IL-10 [7,25]	Inflammatory cytokines, chemokines, hyperglycaemia, lipoproteins, smoking, oxidative stress and generally the inflammation cascade.	Anti-inflammatory agent that opposes the inflammatory sequence. Has a negative feedback mechanism via inflammatory cytokines. There are indications that it reduces the expression of ICAM-1, VCAM-1, IL-1.

CRP – C-reactive protein, ICAM – intercellular adhesion molecule, IL – interleukin, IL-1ra – IL-1 receptor antagonist, MCP – monocyte chemoattractant protein, NF – nuclear factor, TNF – tumour necrosis factor, VCAM – vascular cell adhesion molecule.

particular shear stress, that is, the force exerted by the blood parallel to the vascular wall during pulsatile flow. This stress, depending on how it is applied, causes a corresponding endothelial reaction over a spectrum ranging from the anti-atheromatous (when the stress is steady) to the pro-atheromatous (when the changes in shear stress are abnormal in relation to the anatomy of the vessel).[20]

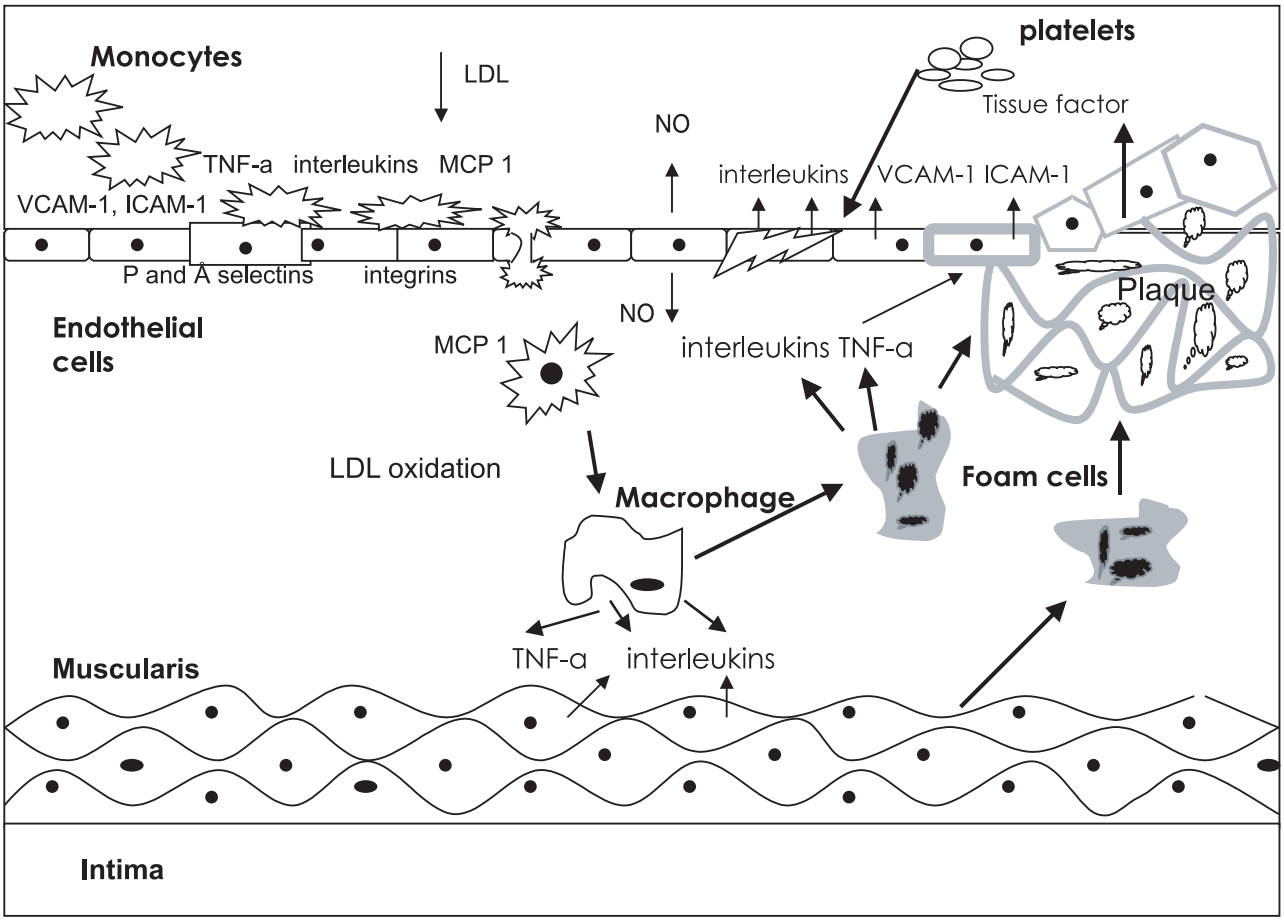

Figure 1. Schematic representation of the process of attraction, adhesion and entry of monocytes beneath the endothelial layer that will ultimately result in the formation of foam cells and atheromatous plaque. (Abbreviations as in Table 1)

Steady shear stress

The chronic application of shear stress, as happens throughout most of the vascular bed, provides the endothelium with continuous haemodynamic stimuli that contribute to the maintenance of homeostasis in the circulation and the vascular anatomy and militate against the inflammatory atheromatous process.[21] The main "atheroprotective" mediators that have been associated with the existence of steady shear stress are as follows:

1. Nitric oxide (NO)

The free radical of NO is a potent molecule that is identical to endothelium derived relaxation factor (EDRF). It is synthesised by L-arginine, with a contribution from the endothelial synthase of NO (eNOS), and its secretion was first reported in 1986.[20] NO has been found to inhibit platelet aggregation and leucocyte adhesion to the endothelium, to reduce the tone and proliferation of smooth muscle cells, and to alter lipoprotein metabolism.[22] Its main action, however, is the reduction of the expression of adhesion molecules such as ICAM-1, vascular cell adhesion molecule (VCAM)-1, and chemokines MCP-1, thus inhibiting leucocyte adhesion and inflammation, while at the same time activating the antioxidative super oxide dismutase (SOD) enzyme system. The action of NO is so fundamental that in experimental models vascular injury during inhibition of its production leads to the onset of the atheromatous process.[23]

2. Prostacyclin (PGI_2)

Its effect is achieved via activation of adenylic cyclase and cyclic adenosine monophosphate (c-AMP). More specifically, it appears to act as a vasodilatory agent (on smooth muscle) and as a powerful antithrombotic (inhibiting platelet aggregation and thrombus formation).[24]

3. Anti-inflammatory cytokines

In addition, the endothelium, in its attempt to moderate the inflammatory reaction, produces and secretes a number of anti-inflammatory cytokines, such as IL-10, transforming growth factor TGF-β and IL-1 receptor antagonist (IL-1ra).[7,25]

Unsteady shear stress. The role of free oxygen radicals

Reactive oxygen species (ROS) comprise a mechanism of intra- and intercellular communication. The balance between the oxidant and antioxidant mechanisms in the cell is very important, because it affects functions such as cytogenesis, gene expression, the secretion of various chemical mediators, and apoptosis. If this balance is tilted in the direction of oxidant mechanisms, as seems to occur in the presence of the classic risk factors, it creates the so-called oxidative stress conditions. Superoxide ($O_2^{\bullet}$) has been found to be the most important agent of intracellular oxidative stress. It inactivates NO (which it neutralises to peroxynitrite $ONOO^{\bullet}$), thus causing endothelial dysfunction, enhancement of the inflammatory process and the initiation of atheromatosis.[7] The NADP/NADPH enzyme system, which is found in foam macrophages, produces the above oxygen radical, while quantities of it are also synthesised by other enzymes, such as the xanthinic oxidase (XO) system and cycloxygenase (COX).[26] A further agent that appears to reduce the concentration of NO is the endogenous eNOS inhibitor known as asymmetric dimethylarginine (ADMA), which has been attracting increasing interest since its levels were associated with the existence of known risk factors.[27,28] It has also been proved that when concentrations of L-arginine or tetrahydrobiopterine cofactor are reduced, then eNOS probably also contributes to ROS production.

In the presence of unsteady shear stress (turbulent flow conditions) the increase in intracellular oxidative stress results in LDL oxidation, inhibition of the defensive SOD system, an increase in the expression of the NF-kB gene and hence the activation of cytokines.

It is also important to mention endothelin-1, which is one of the main vasoconstrictive factors with an action that opposes that of NO and whose secretion has been found to be activated by high shear stress, among other things.[7] Additionally, an increase in angiotensin II also results in an increase in endothelin-1 and in the production of oxygen radicals by the endothelium and by smooth muscle cells,[29] mainly via activation of the NADP/NADPH system.

Finally, oxidative stress, which appears also to be increased by the contribution of a multitude of proinflammatory cytokines, plays a very important role in the process of atheromatosis, which it promotes mainly through the mechanism of endothelial dysfunction.[30] It also appears to participate actively, along with inflammatory mechanisms, in the rupture of atheromatous plaque during acute coronary events and in the subsequent endothelial dysfunction during this phase.[31]

Endothelial function in conditions of infection

Another important parameter is that which occurs in conditions of systemic inflammation in the context of infection. The endotoxin due to infection by Gram(-) bacteria has been found to cause the secretion of TNF-a, IL-1 and IL-6 by monocellular phagocytes.[32] More generally, it has been found that cytokines are produced acutely by external stimuli from endo- and exotoxins, which leads to vasodilation in experimental models. In contrast, it has been found that endothelin production in humans is increased both in systemic acute endotoxinaemia and under conditions of sepsis. There are also studies that have connected the endothelial dysfunction caused by an inflammatory reaction to infective agents with the mechanism that causes atheromatosis and acute ischaemic syndromes. Specifically, injection of *Salmonellae typhi* caused acute endothelial dysfunction.[33] The probability that chronic systemic inflammation contributes to the inflammatory atheromatous process has been mentioned in recent years. It has been reported that chronic infection with *Helicobacter pylori,* by the intracellular microbe *Chlamydia Pneumonia*, by *Epstein-Barr* or cytomegalovirus could lead to atheromatosis via cytokine activation, although prospective epidemiological studies of cardiovascular risk have so far not reached definite conclusions.[34] Finally, it has been established that levels of CRP change in inverse proportion to levels of NO synthesis and that patients with elevated CRP have impaired endothelial function.[2,35]

Anti-inflammatory properties of pharmaceutical agents. Is there a chance of therapeutic intervention?

The assiduous study of the inflammatory cascade in recent years has highlighted the need for therapeutic weapons for the management of the inflammatory atherosclerotic and thrombotic process that is promoted by cytokines.

It is well known that physical exercise is associated with a lower incidence of cardiovascular events. There are also data to suggest that healthy volunteers with high levels of physical activity have 33% lower CRP levels and that an increase in exercise is related with a reduction in inflammation.[36]

It also appears that the Mediterranean diet, especially the lower consumption of saturated fatty acids, is associated with a reduction in adhesion molecules,[37] while moderate alcohol consumption (two glasses per day) is associated with low CRP levels. The dietary intake of antioxidants, such as vitamins C and E, folic acid, arginine, flavonoids, or an increase in their consumption through supplements, appear to reduce LDL oxidation, monocyte attraction, adhesion molecule formation and smooth muscle proliferation, while there are indications that they increase the bioavailability of NO,[38] actions that are opposed to the inflammatory processes of atheromatosis. However, in randomised studies antioxidant treatment has so far not been shown to reduce cardiovascular events or to increase survival. One category of drugs with a significant anti-inflammatory action, apart from their ability to improve lipidaemic profile (pleiotropic actions), are the inhibitors of HMG-coA reductase: the statins. More specifically, it appears that statin administration significantly reduces levels of IL-6, TNF-a and CRP.[39-41] This action is achieved through a reduction in the transcription of NF-kB, which is known to promote the synthesis of proinflammatory cytokines.[41] The anti-inflammatory action of statins seems to be partly responsible for their positive effect on survival, although the precise biochemical mechanisms remain to be investigated.

Converting enzyme inhibitors, especially those with the greatest expression in tissues, also improve endothelial function via an increase in the bioavailability of NO, the secretion of which reduces adhesion molecules, proinflammatory cytokines (IL-8), chemokines (MCP-1) and CRP, while having a beneficial effect on NF-kB.[42,43] In this way they combat the formation of atheromatous plaque and its rupture in acute coronary syndromes. It has also been established that aspirin, a non-selective inhibitor of cycloxygenase, in a dosage of 300 mg, reduces cytokine levels (IL-6, TNF-a and CRP), platelet activation and thrombin production in patients with stable angina[44] or a recent infarction.[45] In addition, the theienopyridines, whose main agent is clopidogrel, in addition to their antiplatelet properties also seem to have an anti-inflammatory action, principally via inhibition of the inflammatory agent CD40 ligand, which is produced by platelet activation.[46,47]

There is much discussion nowadays about selective COX-2 inhibitors (coxibs), which according to the results of experimental studies[48] could lead to a reduction in atherogenesis via a reduction in the production of cytokines like IL-6. In spite of that, their addition to the treatment of arthritics led not to a reduction in cardiovascular events, but to the same or even a higher incidence, casting doubt on this category of drugs.[49] Their main mechanism seems to be the inhibition of the action of the vasodilatory and anti-inflammatory prostacyclin (PGI_2) on platelets, with no restriction of thromboxane TX2.

There also appears to be positive evidence for the intake of ω-3 fatty acids, which by reducing the activation of adhesion molecules in the endothelium and inhibiting COX seem to be associated with a reduced incidence of coronary artery disease.[50]

Tracking down the precise pathological mechanisms involved in inflammation-atheromatosis has been a field of growing research interest during the last two decades. The importance of inflammatory agents is also dictated by the fact that the levels of some of them, such as, for example, P-selectin, serum amyloid-A, ICAM-1, IL-6, CRP and TNF-a, have been associated with an increase in cardiac risk in a number of studies.[2] More specifically, elevated CRP behaves in a way that reflects the extent of the atheromatous process, as well as the extent of myocardial ischaemia and necrosis.[51] Additionally, its levels have been proposed as an index for the monitoring of anti-inflammatory treatment.

It is thus apparent that there is a growing acceptance of the clinical importance of the above agents as factors involved in the entire spectrum of atheromatosis, as well as in cardiovascular morbidity and mortality. This makes the need for successful therapeutic interventions directed against inflammation ever more important.

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