MedCrave
Step into the World of Research

Journal of Cardiology & Current Research

Review Article

Open Access

CrossMark

Inflammatory mediators and their special roles in diverse clinical circumstances

Abstract

The involvement of inflammation is described in all stages of atherosclerosis as well as in dyslipidemias, particularly in lipoproteins (especially oxidized LDL), coronary syndromes, hypertension, diabetes, infections, obesity, and also in the use of sexual replacement hormones. From the first steps of leukocyte recruitment in the nascent atheromatic lesion to the development of atheroma plaque, culminating in its rupture and thrombosis in the acute coronary event, we found a constant release of inflammatory mediators, soluble in plasma, from macrophages, T lymphocytes, endothelial cells and smooth muscle vessels of the vessels, hepatocytes, and adipocytes. The greatest evidence relating inflammation to the future development of cardiovascular events has been verified in large-scale population studies. High concentrations of inflammatory markers such as TNF-α, IL-6, ICAM-1, P-selectin, E-selectin, C Reactive Protein, fibrinogen, and amyloid serum A, in apparently healthy individuals, have shown predictive value for future vascular events. Considering the multifactorial etiology of coronary artery disease and its inflammatory nature, it was possible to find an association between the presence of risk factors and the increase in the concentration of biomarkers of inflammation. TNF-α is a multifunctional cytokine derived from smooth endothelial and muscle cells, as well as macrophages present in the coronary atheroma. It is involved in a number of cardiovascular processes, being increased in congestive heart failure.

Keywords: endothelium, atherosclerosis, coronary artery disease, inflammation markers, interleukins, cytokines

Volume 15 Issue 5 - 2022

Wilson Salgado Filho,[1] Anita LR Saldanha,[1] André Luis Valera Gasparoto,[2] Ana Paula Pantoja Margeotto,[1] Elisa Rinaldi Nunes,[1] Renato Cesar da Silva de Oliveira,[1] Gustavo Costa Pontes,[1] Luiza Ferrari de Castro Melo,[1] Tania Leme da Rocha Martinez[1]

[1]Nephrology Department, BP - A Beneficência Portuguesa de São Paulo, São Paulo, Brazil

[2]Intensive Care Unit, BP - A Beneficência Portuguesa de São Paulo, São Paulo, Brazil

Correspondence: Tania Leme da Rocha Martinez, BP, Rua Comandante Ismael Guilherme, 358 - Jardim Lusitânia, 04031-120 - São Paulo – SP, Brazil, Tel 55 11 98323-9863, Fax 55 11 3842-3789, Email tamar@uol.com.br

Received: December 15, 2022 | **Published:** December 30, 2022

Abbreviations: CAD, coronary artery disease; CRP, C reactive protein; HRT, hormone replacement therapy; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; NO, nitric oxide; TNF-α, tumour necrosis factor-alpha

Introduction

Mediators and markers of inflammation continue to be the most explored points in scientific research linked to atherosclerosis and the resulting vascular diseases. There are many biochemical elements implicated with the inflammation mediators and markers and this review shows their identities and their effects. Cytokines identified as participants in the mechanisms of atherogenesis were first discovered as involved in the process of natural (or innate) and specific (or acquired) immunity, responsible for defending against foreign organisms such as viral or bacterial agents. They are protein hormones produced mainly by mononuclear phagocytes (monocytes) in direct response to microbes or originating from T lymphocytes (lymphocytes) when stimulated by antigens, as part of specific immunity. T lymphocytes produce several cytokines that primarily serve to regulate the activation, growth, and differentiation of the various lymphocyte populations. Other cytokines derived from the T lymphocyte work primarily in the activation and regulation of inflammatory cells, such as mononuclear phagocytes, neutrophils, and eosinophils. Thus, cytokines derived from T lymphocytes are effector molecules of cell-mediated immunity and are also responsible for communications between the cells of the immune and inflammatory systems.

Originally, Tumour Necrosis Factor-alpha (TNF-α) was identified as a factor present in the plasma of animals exposed to Lipopolysaccharide (LPS), being able to cause hemorrhagic necrosis of tumors, and therefore thus referred to as tumor necrosis factor. At low concentrations, LPS stimulates the functions of mononuclear phagocytes and acts as a polyclonal activator of B-lymphocytes translating host responses that contribute to the elimination of invasive bacteria. In the presence of severe gram-negative bacterial infections, high concentrations of LPS occur that can cause tissue injury, disseminated intravascular coagulation, and shock, often resulting in death. It is currently clear that TNF-α one of the main mediators of these LPS effects. However, most biological actions of TNF-α occur at low concentrations, acting locally as a paracrine and autocrine regulator of leukocytes and endothelial cells. TNF-α causes endothelial cells to become adhesive to leukocytes, initially for neutrophils, and subsequently to monocytes and lymphocytes.

These actions contribute to the accumulation of leukocytes in localized inflammatory sites. TNF-α stimulates mononuclear phagocytes and other cell types to produce cytokines, such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), TNF-α itself, and low molecular weight inflammatory cytokines of the Interleukin-8 family (IL-8). TNF- α an endogenous pyrogen that acts on cells in the hypothalamic regulatory regions of the brain, inducing the onset of fever. Its production in response to TNF-α (and also IL-1) is mediated by an increase in prostaglandin synthesis by cytokine-stimulated hypothalamic cells. Prostaglandin synthesis inhibitors, such as aspirin, reduce fever by blocking this action of TNF-α or IL-1. TNF-α activates the coagulation system, mainly by altering the balance between the procoagulant and anticoagulant properties of the endothelium. Prolonged systemic administration of TNF-α in experimental animals causes metabolic changes in cachexia, a state characterized by depletion of muscle and adipose cells. Two mechanisms are proposed for the triggering of cachexia: (a) direct suppression of appetite for TNF-α; (b) inhibition of the synthesis of lipoprotein lipase, an enzyme necessary for the release of fatty acids from circulating lipoproteins, preventing its use as an energy source by tissues and leading to exaggerated protein catabolism present in cachexia. Although TNF-α alone can cause cachexia in experimental

animals, other cytokines such as IL-1 may also contribute to this state that accompanies certain diseases such as cancer. TNF-α and also IL-1 and IL-6, act on hepatocytes by increasing the synthesis of certain proteins such as amyloid protein Serum A, C Reactive Protein (CRP) and fibrinogen, constituting the so-called acute phase response to inflammatory stimuli. Although the physiological role of acute phase response is not completely known, it is assumed that it favors the efficacy of specific immunity. A combination of fever, elevated IL-6 levels, elevation of acute phase reagents, bone marrow suppression, and coagulation activation were observed in patients treated with intravenous TNF-α as part of antineoplastic chemotherapy. TNF-α depresses myocardial contractility by an as yet unknown mechanism.

Interleukin-1: it was first defined as a polypeptide derived from mononuclear phagocytes, which constitute its main cellular source, activated by bacterial products such as LPS, by cytokines derived from macrophages such as TNF-α or IL-1 itself. The synthesis of IL-1 differs from the synthesis of TNF-α by its origin of distinct cell types, such as epithelial and endothelial cells, which provide potential local sources of IL-1 in the absence of macrophage-rich infiltrates. The production of IL-1 by the endothelial cell is also increased by mechanical stimulation such as "shear stress" and infectious agents (bacterial, viral, or parasitic).[1,2] Endothelial cell infection with Trypanosoma Cruzi, for example, has been shown to increase IL-1 expression by these cells, suggesting a potential role of cytokines secreted by endothelium in the pathogenesis of Chagas Disease.[3] In many cell types, IL-1 causes the synthesis of enzymes that produce prostaglandins, potentially capable of causing fever when at high concentrations. At first, it was very surprising to note the extensive analogies of IL-1 actions with those of TNF-α, a striking example of redundancy of the effects of cytokines. However, there are several important differences between these cytokines. First of all, IL-1 does not produce a per se muscle lesion, although it is secreted in response to LPS, which can potentiate the weight injury caused by TNF-α. Moreover, even in the face of very high systemic concentrations, IL-1 is not lethal. IL-1 seems to be more potent than TNF-α as a stimulator of IL-6 production, both participating in the regulation of acute phase protein liver synthesisdescribed below.

Interleukin-6: is a cytokine synthesized by mononuclear phagocytes, endothelial cells, fibroblasts, some activated T lymphocytes, and smooth muscle cells in response to IL-1 and, to a lesser extent, TNF-α. The two best-described actions of IL-6 occur in B-lymphocytes and hepatocytes:

a) IL-6 serves as the main growth factor for activated B-lymphocytes, as well as following its differentiation process. IL-6 acts similarly as a growth factor for many plasmocytomas or myelomas. In addition, IL-6 can serve as a co-stimulator of T lymphocytes and thymocytes.

b) IL-6 causes hepatocytes to synthesize several plasma proteins that contribute to the so-called acute phase response, of greater interest to CRP because it is associated with various cardiovascular diseases.

C-Reactive Protein

The acute phase response is an important pathophysiological phenomenon that accompanies inflammation.[4,5] Attention to this phenomenon occurred for the first time with the discovery of high concentrations of CRP during the acute phase of pneumococcal pneumonia, so called for reacting with pneumococcal polysaccharide C.[6] Since then, such systemic changes have been reported as an acute phase response[7] although they also follow chronic inflammatory states. CRP can occur in association with a wide variety of diseases, including infections, trauma, infarction, inflammatory arthritis, various neoplasms, and many cardiovascular diseases. CRP influences one or more stages of inflammation. One of the main accepted functions of CRP is its ability to bind to phosphocholine, allowing the recognition of foreign pathogens and activating the complement and/or binding system of phagocytes, suggesting that it can initiate the elimination of target cells by interacting with both humoral and cellular intersection systems at the site of inflammation. The speed of the CRP response, in contrast to the slow immune adaptive response expressed by antibody production, indicates that CRP is a component of the innate immune response.[8] During inflammatory states, CRP presents increases of at least 25%, constituting a positive acute phase protein (as well as fibrinogen and serum amyloid A), unlike others that can decrease its concentrations, called negative acute phase proteins such as albumin and transferrin.[9] Such changes are mainly the reflection of its production in hepatocytes under the influence of cytokines such as TNF-α, IL-1, and IL-6, the latter seeming to be the main inducer of most acute phase proteins in the liver.[10,11]

Despite the lack of diagnostic specificity, the measurement of serum levels of acute phase proteins is useful because it can quantify the intensity of an inflammatory process.

C-Reactive protein in cardiovascular diseases

Several studies have shown that serum CRP concentration may correlate with the prognosis of cardiovascular disease, constituting an additional risk factor in unstable angina and myocardial infarction, when at high concentrations.[12,13] The mechanisms responsible for the association between CRP and cardiovascular disease are unclear. CRP may be only a marker of inflammation and thrombotic risk, without a specific role degree of atherosclerosis, or may have a direct effect suggested by the following observations:

a) CRP has been found in atherosclerotic lesions

b) Pro-inflammatory effects of CRP include induction of inflammatory cytokines and tissue factor[14,15] resulting in tissue damage in some situations. This was illustrated by the approximately 40% increase in the extent of experimental myocardial infarction in rodents when human CRP was infused in the peri-infarction period.[16] Among the cytokines induced by CRP, the production of chemokine MCP-1 (Monocyte Chemoattractant Protein-1) in human endothelial cells was evidenced, influencing the local inflammatory response in the atherosclerotic plaque by the recruitment of monocytes and lymphocytes.[17,18]

c) CRP binds to low-density lipoprotein (LDL) allowing this particle to be captured by macrophages without the need for modification to the oxidized form.[19]

Women have higher concentrations of CRP than men[20] and the predictive value of CRP for cardiovascular disease also applies to females.[21,22] Women receiving hormone replacement therapy (HRT) have higher levels of CRP than those without replacement, suggesting a pro-inflammatory effect of HRT. In the PEPI (Postmenopausal Estrogen/Progestin Interventions) study, CRP concentrations were measured in 365 women at baseline and at 12 and 36months using HRT.[23] Compared to placebo, all HRT preparations were associated with an increase in serum CRP, which occurred mainly during the first year of treatment, with levels 85% higher than baseline. Similar data were obtained from the Women's Health Study, which found CRP concentrations twice as high in women receiving HRT, compared to those without HRT.[24]

Intercellular adhesion molecule-1 (ICAM-1)

Both in experimental and clinical studies, it is well documented that the involvement of circulating leukocytes, specifically monocytes,

in the vascular wall is an essential phenomenon in the inflammatory response.[25] The induction of different cell-adding molecules on the endothelial surface, such as ICAM-I and selectins, seems to be the earliest event in atherogenesis.

ICAM-1 is a prototype protein of the immunoglobulin superfamily. It is produced by endothelium cells, fibroblasts, epithelial cells, and hematopoietic cells and its expression is intensified by cytokines such as TNF-α, IL-1, and lipoproteins. The increase in ICAM-1 expression was associated with significant monocyte and T-lymphocyte recruitment in the subendothelial region,[26] acting as a link with the integrins present on the leukocyte surface, during the phase of firm leukocyte strain to the endothelium, which occurs after the rolling and seathing process.

E-selectin and P-selectin

E-selectin and P-selectin, belonging to the superfamily of selectins, were described according to the type of cell initially found: E-selectin (endothelium) and P-selectin (platelets). Unlike other binding molecules, which bind to other proteins, selectins interact with carbohydrates from both leukocytes and endothelial cells. E-selectin is exclusively expressed by endothelial cells that have been stimulated by TNF-α, IL-1, or endotoxins (LPS) cytokines, and immunocomplex aggregates. E-selectin binds to the glycoproteins present on the surface of leukocytes, promoting its adhesion to the endothelial surface.[27] P-selectin is found in unstimulated platelet alpha granules and Weibel-Palade corpuscles of endothelial cells. On activation, triggered by thrombin and histamine, P-selectin is rapidly redistributed on the surface of platelets and endothelial cells. It has been observed that P-selectin is able to bind to various types of leukocytes, including neutrophils and monocytes, thus mediating platelet-leukocyte and endotheliocyte cell interactions.

Cytokines as biomarkers of the atherosclerosis process

Inflammation involvement is described in all stages of atherosclerosis. From the first steps of leukocyte recruitment in the nascent atherosclerotic lesion to the development of atheroma plaque, culminating in its rupture and thrombosis in the acute coronary event, we found a constant release of inflammatory mediators, soluble in plasma, from macrophages, T lymphocytes, endothelial cells and smooth muscle vessels of the vessels, hepatocytes, and adipocytes.[4,21,28] The greatest evidence relating inflammation to the future development of cardiovascular events has been verified in large-scale population studies. High concentrations of inflammatory markers such as TNF-α, IL-6, ICAM-1, P-selectin, E-selectin, CRP, fibrinogen, and amyloid Serum A, in apparently healthy individuals, have shown predictive value for future vascular events.[21,29–37] Considering the multifactorial etiology of coronary artery disease (CAD) and its inflammatory nature, it was possible to find an association between the presence of risk factors and the increase in the concentration of biomarkers of inflammation.

TNF-α is a multifunctional cytokine derived from smooth endothelial and muscle cells, as well as macrophages present in the coronial atheroma. It is involved in a series of cardiovascular processes, being increased in congestive heart failure, and may produce cardiomyopathy, left ventricular dysfunction, and pulmonary edema. TNF-α levels were measured in patients with CAD participating in the CARE study, on average 8.9months after the initial myocardial infarction. In the whole group, TNF-α levels were significantly higher among patients, compared with controls (2.84 versus 2.57pg/mL, p=0.02). The excessive risk of recurrence of coronary events after myocardial infarction was predominantly observed in those with higher levels of TNF-α, i.e., above 4.17pg/mL (95th percentile of the control distribution), who presented a risk approximately 3 times higher (relative risk = 2.7; confidence interval (95%) 1.4-5.2; p=0.004) (Adapted from Ridker et al.).[30]

Oxidized LDL and inflammation - the LDL retained in the intima partly linked to proteoglycans, is subjected to oxidative modifications that induce the expression of binding molecules, chemokines, pro-inflammatory cytokines, and other mediators of inflammation in macrophages and vascular wall cells.[38–40]

Dyslipidemia and inflammation - other lipoprotein particles such as very low density (VLDL) and intermediate-sized particles (IDL), also have considerable atherogenic potential and can be modified in oxidized form and activate inflammatory state in endothelial cells.[41,42] High-density lipoproteins (HDL) protect against atherosclerosis by the mechanism of reverse transport and by its antioxidant action. HDL particles carry antioxidant enzymes such as paraoxonese neutralizing the pro-inflammatory effects of oxidized particles.[28]

Hypertension and inflammation - angiotensin II, in addition to its vasoconstrictor properties can stimulate the production of IL-6, VCAM-1, and MCP-1 in smooth endothelial and muscle cells.[43–45]

Diabetes and inflammation - hyperglycemia can lead to macromolecule modification resulting in advanced final glycation products[46] that increase the expression of cytokines and other inflammatory markers in endothelial cells.[47]

Obesity and inflammation - visceral obesity, regardless of the effects on insulin resistance and lipoproteins, has pro-inflammatory action due to the production of cytokines such as TNF-α and IL-6 by adipocytes.[48] Infection and inflammation - infectious agents in endothelial cells may produce inflammatory stimulation that accentuates atherogenesis, such as Chlamydia pneumoniae found not rarely in human atheroma plaques.[49-51]

Inflammation in acute coronary syndromes

The process of chronic low-intensity vascular inflammation, present throughout the natural history of atherogenesis, can become intensified with the worsening of the disease, clinically externalizing in the form of stable, unstable, and acute coronary syndromes. High concentrations of circulating inflammatory markers such as IL-6, CRP, and serum amyloid-A often accompany these conditions, particularly in acute coronary syndromes.[52–59] However, the intensity of inflammation not only reveals an increased risk of infarction but also participates in the precipitation of coronary events promoting thrombus formation due to an increase in other acute phase proteins besides CRP, such as fibrinogen. In fact, the elevation of CRP in unstable patients does not seem to be merely related to the extent and severity of atherosclerosis, since only 20% of patients with stable angina and a high incidence of multi-arterial CAD have high concentrations of CRP compared to 70% of patients with unstable angina.[53] In addition, CRP concentrations in patients with severe peripheral vascular insufficiency and indication for revascularization do not differ significantly from those observed in patients with symptomatic uniarterial CAD.[60] Practically all patients with infarction preceded by unstable angina have elevated CRP, and the acute event may result from the coexistence of a prothrombotic state and exaggerated coronary vasoreactivity associated with a higher vascular inflammatory state. The comparative study of concentrations of CRP, serum amyloid A, IL-6, and soluble ICAM-1 revealed significantly higher values in patients with CAD in relation to controls but did not correlate with the severity of the disease, suggesting that these markers may better reflect the extent of the arterial inflammatory process than the degree of localized obstructions of coronary lesions.[61]

Citation: Filho WS, Saldanha ALR, Gasparoto ALV, et al. Inflammatory mediators and their special roles in diverse clinical circumstances. *J Cardiol Curr Res.* 2022;15(5):133–138. DOI: 10.15406/jccr.2022.15.00568

Sex hormones and cardiovascular disease

The disparity between the incidence of CAD in premenopausal women and men of the same age suggests that endogenous sex hormones such as estradiol, progesterone, and/or androgens have an important impact on the atherogenesis process.[62]

Vascular biological effects of female sex hormones

Progesterone has often been described as having effects opposite to estradiol, but such an impression may represent an exaggerated simplification. For example, progesterone in the uterus inhibits endometrial growth induced by estradiol, but in the breast, both estradiol and progesterone, promote breast tissue growth.[63–65] Experimental studies have suggested that the addition of progesterone could prevent increases in nitric oxide (NO) circulating during HTR.[66] However, no difference was found in the response of increased flow to reactive hyperemia (endothelium-dependent) that reflects NO production in women receiving estradiol alone or in association with progesterone.[67]

Vascular biological effects of male sex hormones

The approximately 10-year interval between the clinical manifestation of CAD between men and women is usually attributed to the possible anti-atherogenic effect of female sex hormones.[68] The mechanisms by which the biological effects of estradiol at the level of estrogen receptors are mediated, present in the smooth muscle cells of vessels and endothelial cells, have been extensively studied.[69–77]

Estradiol acts on various functions of smooth muscle cells, including contractility and growth. Some of the vasodilator effects of estradiol are endothelium-dependent, determining increased production and release of NO by endothelial cells, or acting directly on the smooth muscle cells of the vessels with urn action similar to that of calcium channel blockers. Estradiol attenuates vasoconstriction mediated by angiotensin II and nor-epinephrine, substances that primarily release Ca++ ions from intracellular stocks, and may also produce vasodilatation by activating potassium channels. Some anti-atherogenic effects of estradiol are partly attributed to the inhibition of growth and proliferation of smooth muscle cells.[78-80] Observational studies in pre-and postmenopausal women reveal that physiological concentrations of estradiol increase acetylcholine-mediated vasodilatation in the coronary circulation.[81-83] Progesterone, like estradiol, acts by binding to specific receptors that have not been found in the ascending aorta, internal carotid arteries, coronary arteries, and left atrial appendage. However, receptors have not been identified in the vessels of the human uterus and breast, prostate, kidneys, and gastrointestinal tract. In the internal and coronary carotid arteries, progesterone receptors were located in the nuclei of the endothelial cells and in the vascular canals within atherosclerotic plaques. These findings suggest that the heart and large vessels are target organs for steroid hormones.[84] Much less is known about the vascular biological effects of androgens and their influence on the process of atherogenesis, compared to the extensive volume of accumulated information on the action of female sex hormones. Experimental studies have shown that testosterone supplementation in orquiedectomized mice with a hypercholesterolemic diet reduced the extent of fatty stria in the aorta in relation to the control group. However, this testosterone attenuating effect has not been observed in orquiedectomized animals with androgenic supplementation treated with a selective aromatase inhibitor enzyme that converts testosterone into estradiol. The administration of estradiol to orquiedectomized males attenuated the injury formation to the same degree as testosterone administration. The results indicated that testosterone can attenuate atherogenesis at its early stage mainly by its conversion into estradiol by means of the aromatase enzyme, present in the vessel wall.[85]

Inflammatory markers in percutaneous coronary interventions

Coronary angioplasty by balloon, rotational atherectomy stent implantation, and other forms of percutaneous coronary interventions are recognized as clinical models of inflammation because they lead to the release of inflammatory mediators because of endothelial aggression and plaque rupture, besides producing a brief tissue ischemia.[86] Thus, the use of percutaneous coronary interventions in patients with stable coronary syndromes, often linked to a little intense inflammatory state, can increase the expression of myocardial infarction to varying degrees and favor the study of possible modulating effects of certain substances on inflammation present in atherosclerotic disease. Considering the evidence based on numerous studies on HRT, that sex steroids may play a role in regulating inflammatory responses involved in atherogenesis[23,24,87,88] it is feasible to study possible modulating effects of male and female hormones on the expression of myocardial infarction associated with percutaneous coronary interventions.

Acknowledgments

None.

Conflicts of interest

No conflicts of interest.

Funding

None.

References

1. Sterpetti AV, Cucina A, Morena AR, et al. Shear stress increases the release of interleukin–1 and interleukin–6 by aortic endothelial cells. *Surgery*. 1993;114(5):911–914.
2. Woodroffe SB, Garnett HM, Danis VA. Interleukin–1 production and cell–activation response to cytomegalovirus infection of vascular endothelial cells. *Arch Virol*. 1993;133(3–4):295–308.
3. Tanowitz HB, Gumprecht JP, Spurr D, et al. Cytokine gene expression of endothelial cells infected with Trypanosoma cruzi. *J Infect Dis*. 1992;166(3):598–603.
4. Kushner I. The phenomenon of the acute phase response. *Ann N Y Acad Sci*. 1982;389(1):39–48.
5. Gabay C, Kushner I. Acute–phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340(6):448–454.
6. Tillett WS, Francis T. Serological reactions in pneumonia with a non–protein somatic fraction of pneumococcus. *J Exp Med*. 1930;52(4):561–571.
7. Kushner I. Regulation of the acute phase response by cytokines. *Perspect Biol Med*. 1993;36(4):611–622.
8. Hoffmann JA, Kafatos FC, Janeway CA, et al. Phylogenetic perspectives in innate immunity. *Science*. 1999;284(5418):1313–1318.
9. Morley JJ, Kushner I. Serum C–reactive protein levels in disease. *Ann N Y Acad Sci*. 1982;389(1):406–418.
10. Gauldie J, Richards C, Harnish D, et al. Interferon beta 2/B–cell stimulatory factor type 2 shares identity with monocyte–derived hepatocyte–stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci USA*. 1987;84(20):7251–7255.

11. Woods A, Brull DJ, Humphries SE, et al. Genetics of inflammation and risk of coronary artery disease:the central role of interleukin–6. *Eur Heart J*. 2000;21(19):1574–1583.
12. Kervinen H, Palosuo T, Manninen V, et al. Joint effects of C–reactive protein and other risk factors on acute coronary events. *Am Heart J*. 2001;141(4):580–585.
13. Ridker PM. High–sensitivity C–reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*. 2001;103(13):1813–1818.
14. Ballou SP, Lozanski G. Induction of inflammatory cytokine release from cultured human monocytes by C–reactive protein. *Cytokine*. 1992;4(5):361–368.
15. Cermak J, Key NS, Bach RR, et al. C–reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood*. 1993;82(2):513–520.
16. Griselli M, Herbert J, Hutchinson WL, et al. C–reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med*. 1999;190(12):1733–1740.
17. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C–reactive protein on human endothelial cells. *Circulation*. 2000;102(18):2165–2168.
18. Pasceri V, Cheng JS, Willerson JT, et al. Modulation of C–reactive protein–mediated monocyte chemoattractant protein–1 induction in human endothelial cells by anti–atherosclerosis drugs. *Circulation*. 2001;103(21):2531–2534.
19. Zwaka TP, Hombach V, Torzewski J. C–reactive protein–mediated low density lipoprotein uptake by macrophages:implications for atherosclerosis. *Circulation*. 2001;103(9):1194–1197.
20. Garcia–Moll X, Zouridakis E, Cole D, et al. C–reactive protein in patients with chronic stable angina:differences in baseline serum concentration between women and men. *Eur Heart J*. 2000;21(19):1598–1606.
21. Ridker PM, Buring JE, Shih J, et al. Prospective study of C–reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation*. 1998;98(8):731–733.
22. Ridker PM, Hennekens CH, Buring JE, et al. C–reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342(12):836–843.
23. Cushman M, Legault C, Barrett–Connor E, et al. Effect of postmenopausal hormones on inflammation–sensitive proteins:the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation*. 1999;100(7):717–722.
24. Ridker PM, Hennekens CH, Rifai N, et al. Hormone replacement therapy and increased plasma concentration of C–reactive protein. *Circulation*. 1999;100(7):713–716.
25. Price DT, Loscalzo J. Cellular adhesion molecules and atherogenesis. *Am J Med*. 1999;107(1):85–97.
26. Watanabe T, Fan J. Atherosclerosis and inflammation mononuclear cell recruitment and adhesion molecules with reference to the implication of ICAM–1/LFA–1 pathway in atherogenesis. *Int J Cardiol*. 1998;66(Suppl 1):S45–S53; discussion S55.
27. Hu Y, Kiely JM, Szente BE, et al. E–selectin–dependent signaling via the mitogen–activated protein kinase pathway in vascular endothelial cells. *J Immunol*. 2000;165(4):2142–2148.
28. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105(9):1135–1143.
29. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336(14):973–979.
30. Ridker PM, Rifai N, Pfeffer M, et al. Elevation of tumor necrosis factor–alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation*. 2000;101(18):2149–2153.
31. Ridker PM, Rifai N, Stampfer MJ, et al. Plasma concentration of interleukin–6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000;101(15):1767–1772.
32. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin–6 and C–reactive protein levels with mortality in the elderly. *Am J Med*. 1999;106(5):506–512.
33. Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM–1, ICAM–1, and E–selectin in carotid atherosclerosis and incident coronary heart disease cases:the Atherosclerosis Risk In Communities (ARIC) study. *Circulation*. 1997;96(12):4219–4225.
34. Haverkate F, Thompson SG, Pyke SD, et al. Production of C–reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet*. 1997;349(9050):462–466.
35. Kuller LH, Tracy RP, Shaten J, et al. Relation of C–reactive protein and coronary heart disease in the MRFIT nested case–control study. Multiple Risk Factor Intervention Trial. *Am J Epidemiol*. 1996;144(6):537–547.
36. Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease:prospective study and updated meta–analyses. *BMJ*. 2000;321(7255):199–204.
37. Koenig W, Sund M, Fröhlich M, et al. C–Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle–aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation*. 1999;99(2):237–242.
38. Berliner J, Leitinger N, Watson A, et al. Oxidized lipids in atherogenesis:-formation, destruction and action. *Thromb Haemost*. 1997;78(1):195–199.
39. Williams KJ, Tabas I. The response–to–retention hypothesis of atherogenesis reinforced. *Curr Opin Lipidol*. 1998;9(5):471–474.
40. Witztum JL, Berliner JA. Oxidized phospholipids and isoprostanes in atherosclerosis. *Curr Opin Lipidol*. 1998;9(5):441–448.
41. Dichtl W, Nilsson L, Goncalves I, et al. Very low–density lipoprotein activates nuclear factor–kappaB in endothelial cells. *Circ Res*. 1999;84(9):1085–1094.
42. Mackness MI, Mackness B, Durrington PN, et al. Paraoxonase and coronary heart disease. *Curr Opin Lipidol*. 1998;9(4):319–324.
43. Griendling KK, Fukai MU, Lassègue B, et al. Angiotensin II signaling in vascular smooth muscle. New concepts. Hypertension. 1997;29(1):366–370.
44. Kranzhöfer R, Schmidt J, Pfeiffer CA, et al. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 1999;19(7):1623–1629.
45. Tummala PE, Chen XL, Sundell CL, et al. Angiotensin II induces vascular cell adhesion molecule–1 expression in rat vasculature:A potential link between the renin–angiotensin system and atherosclerosis. *Circulation*. 1999;100(11):1223–1239.
46. Schmidt AM, Yan SD, Wautier JL, et al. Activation of receptor for advanced glycation end products:a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res*. 1999;84(5):489–497.
47. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications:a new perspective on an old paradigm. *Diabetes*. 1999;48(1):1–9.
48. Yudkin JS, Stehouwer CD, Emeis JJ, et al. C–reactive protein in healthy subjects:associations with obesity, insulin resistance, and endothelial dysfunction:a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*. 1999;19(4):972–978.
49. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis:an assessment of the evidence and need for future research. *Circulation*. 1997;96(11):4095–4103.

50. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease:is there a link? *Lancet*. 1997;350(9075):430–436.

51. Kol A, Bourcier T, Lichtman AH, et al. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J Clin Invest*. 1999;103(4):571–577.

52. Berk BC, Weintraub WS, Alexander RW. Elevation of C–reactive protein in "active" coronary artery disease. *Am J Cardiol*. 1990;65(3):168–172.

53. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C–reactive protein and serum amyloid a protein in severe unstable angina. *N Engl J Med*. 1994;331(7):417–424.

54. Toss H, Lindahl B, Siegbahn A, et al. Prognostic influence of increased fibrinogen and C–reactive protein levels in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *Circulation*. 1997;96(12):4204–4210.

55. Rebuzzi AG, Quaranta G, Liuzzo G, et al. Incremental prognostic value of serum levels of troponin T and C–reactive protein on admission in patients with unstable angina pectoris. *Am J Cardiol*. 1998;82(6):715–719.

56. Biasucci LM, Liuzzo G, Grillo RL, et al. Elevated levels of C–reactive protein at discharge in patients with unstable angina predict recurrent instability. *Circulation*. 1999;99(7):855–860.

57. Biasucci LM, Liuzzo G, Colizzi C, et al. Clinical use of C–reactive protein for the prognostic stratification of patients with ischemic heart disease. *Ital Heart J*. 2001;2(3):164–171.

58. Morrow DA, Rifai N, Antman EM, et al. Serum amyloid A predicts early mortality in acute coronary syndromes:A TIMI 11A substudy. *J Am Coll Cardiol*. 2000;35(2):358–362.

59. Heeschen C, Hamm CW, Bruemmer J, et al. Predictive value of C–reactive protein and troponin T in patients with unstable angina:a comparative analysis. CAPTURE Investigators. Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment trial. *J Am Coll Cardiol*. 2000;35(6):1535–1542.

60. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis:a comparison of C–reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA*. 2001;285(19):2481–2485.

61. Rifai N, Joubran R, Yu H, et al. Inflammatory markers in men with angiographically documented coronary heart disease. *Clin Chem*. 1999;45(11):1967–1973.

62. Hanes DS, Weir MR, Sowers JR. Gender considerations in hypertension pathophysiology and treatment. *Am J Med*. 1996;101(3A):10S–21S.

63. Samsioe G. The endometrium: effects of estrogen and estrogen–progestogen replacement therapy. *Int J Fertil Menopausal Stud*. 1994;39(Suppl 2):84–92.

64. King RJB. Estrogen and progestin effects in human breast carcinogenesis. *Breast Cancer Res Tr*. 1993;27:3–15.

65. Shi YE, Liu YE, Lippman ME, et al. Progestins and antiprogestins in mammary tumour growth and metastasis. *Hum Reprod*. 1994;9(Suppl 1):162–173.

66. Rosselli M, Imthurn B, Keller PJ, et al. Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17 beta–estradiol and norethisterone acetate. A two–year follow–up study. *Hypertension*. 1995;25(4 Pt 2):848–853.

67. McCrohon JA, Adams MR, McCredie RJ, et al. Hormone replacement therapy is associated with improved arterial physiology in healthy post–menopausal women. *Clin Endocrinol (Oxf)*. 1996;45(4):435–441.

68. Von Eckardstein A. In: Azziz RA, Nestler JE, Dewailly D, editor. *Androgen Excess Disorders in Women*. 2nd edn. Philadelphia, Lippincott–Raven Publishers; 1997:230.

69. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science*. 1988;240(4854):889–895.

70. McGill HC Jr, Sheridan PJ. Nuclear uptake of sex steroid hormones in the cardiovascular system of the baboon. *Circ Res*. 1981;48(2):238–244.

71. Colburn P, Buonassisi V. Estrogen–binding sites in endothelial cell cultures. *Science*. 1978;201(4358):817–819.

72. Bayard F, Clamens S, Meggetto F, et al. Estrogen synthesis, estrogen metabolism, and functional estrogen receptors in rat arterial smooth muscle cells in culture. *Endocrinology*. 1995;136(4):1523–1529.

73. Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation*. 1994;89(5):1943–1950.

74. Losordo DW, Kearney M, Kim EA, et al. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. *Circulation*. 1994;89(4):1501–1510.

75. Knauthe R, Diel P, Hegele–Hartung C, et al. Sexual dimorphism of steroid hormone receptor messenger ribonucleic acid expression and hormonal regulation in rat vascular tissue. *Endocrinology*. 1996;137(8):3220–3227.

76. Campisi D, Cutolo M, Carruba G, et al. Evidence for soluble and nuclear site I binding of estrogens in human aorta. Atherosclerosis. 1993;103(2):267–277.

77. Lin AL, Gonzalez R Jr, Carey KD, et al. Gender and baboon aortic steroid hormone receptors. *Arteriosclerosis*. 1987;7(3):248–255.

78. Fischer–Dzoga K, Wissler RW, Vesselinovitch D. The effect of estradiol on the proliferation of rabbit aortic medial tissue culture cells induced by hyperlipemic serum. *Exp Mol Pathol*. 1983;39(3):355–363.

79. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. *FASEB J*. 1996;10(5):615–624.

80. Moraghan T, Antoniucci DM, Grenert JP, et al. Differential response in cell proliferation to beta estradiol in coronary arterial vascular smooth muscle cells obtained from mature female versus male animals. *Endocrinology*. 1996;137(11):5174–5177.

81. Gilligan DM, Badar DM, Panza JA, et al. Acute vascular effects of estrogen in postmenopausal women. *Circulation*. 1994;90(2):786–791.

82. Gilligan DM, Quyyumi AA, Cannon RO 3rd. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. *Circulation*. 1994;89(6):2545–2551.

83. Sudhir K, Jennings GL, Funder JW, et al. Estrogen enhances basal nitric oxide release in the forearm vasculature in perimenopausal women. *Hypertension*. 1996;28(3):330–334.

84. Ingegno MD, Money SR, Thelmo W, et al. Progesterone receptors in the human heart and great vessels. *Lab Invest*. 1988;59(3):353–356.

85. Nathan L, Shi W, Dinh H, et al. Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase. *Proc Natl Acad Sci USA*. 2001;98(6):3589–3593.

86. Serrano CV Jr, Ramires JA, Venturinelli M, et al. Coronary angioplasty results in leukocyte and platelet activation with adhesion molecule expression. Evidence of inflammatory responses in coronary angioplasty. *J Am Coll Cardiol*. 1997;29(6):1276–1283.

87. Scarabin PY, Alhenc–Gelas M, Oger E, et al. Hormone replacement therapy and circulating ICAM–1 in postmenopausal women. A randomised controlled trial. *Thromb Haemost*. 1999;81(5):673–675.

88. Oger E, Alhenc–Gelas M, Plu–Bureau G, et al. Association of circulating cellular adhesion molecules with menopausal status and hormone replacement therapy. Time–dependent change in transdermal, but not oral estrogen users. *Thromb Res*. 2001;101(2):35–43.