Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *Innate Inflammation and Stroke*

Pharmacological modulation of vascular inflammation in atherothrombosis

Raffaele De Caterina,[1,2] Marika Massaro,[3] Egeria Scoditti,[3] and Maria Annunziata Carluccio[3]

[1]Institute of Cardiology, "G. d'Annunzio" University, Chieti, Italy. [2]Fondazione "G. Monasterio," Pisa, Italy. [3]CNR Institute of Clinical Physiology, Lecce, Italy

Address for correspondence: Raffaele De Caterina, M.D., Ph.D., Institute of Cardiology, "G. d'Annunzio" University, Chieti, C/o Ospedale SS. Annunziata, Via dei Vestini, 66013 Chieti, Italy. rdecater@unich.it

Vascular inflammation, especially at the level of endothelial cells, has been shown to play a pivotal role in the inception, progression, and clinical complications of atherosclerosis. The common denominators for the activation of inflammatory genes appear to be a small subset of transcription factors—among which include nuclear factor-κB, activator protein-1 (AP-1), and GATA—that function as the central hub of vascular inflammation. Strategies directed to inhibit both the secondary mediators and the primary triggers (atherosclerosis risk factors) appear viable to inhibit atherosclerosis. However, attempts have now been made to address the central hub of vascular inflammation. "Old" drugs, such as dipyridamole, can also now be revisited for properties related to inhibition of vascular inflammation, probably by acting on the common hub of inflammation.

Keywords: inflammation; endothelial cells; endothelial activation; nuclear factor-κB; dipyridamole

Introduction

Our knowledge on the pathophysiology of myocardial infarction and of atherosclerotic vascular disease in general has gradually accrued over the past two centuries but has received an important acceleration in the past 20 years. While myocardial infarction, since its first descriptions, has been linked to coronary artery disease, the instabilization of an otherwise smoldering disease—leading to acute coronary syndromes—has been only the product of knowledge of recent years. Today, "inflammation," in its broader sense, can comprehensively account for the pathogenesis of vascular disease, including its late clinical manifestations. Here, we will briefly review recent knowledge in this area and highlight how even "old fashioned" drugs may need a modern revisitation for properties previously unsuspected, ultimately yielding potential new tools to combat the disease of the past and the present century.

On the origin of myocardial infarction and arterial instabilization

About 40 years ago, knowledge on the origin and pathophysiology of atherosclerosis mostly revolved on the entry of cholesterol esters into vascular intima and on the correlation of such accumulation with hemodynamic stress. Several studies had already indeed correlated high levels of blood cholesterol with atherosclerotic vascular disease. Other studies had correlated the specific location of atherosclerotic plaques in bifurcation and branching points with the shear stress caused by the turbulence of blood flow, considered responsible for the increased endothelial permeability.[1–3] The transport of cholesterol in a free form through endothelial cells and its deposition in intimal phagocytes was being recognized, and gradually the role of low-density lipoprotein (LDL) cholesterol gradually emerged as the principal trigger of atherosclerosis.[4–6] The body of knowledge acquired in those years offered a reasonable interpretation on the origin and progression of atherosclerosis in relation to the well-appreciated risk factors for ischemic heart disease. But those years were also witnessing a dispute, now resolved and forgotten by most, on the pathogenesis of myocardial infarction on an atherosclerotic substrate: important schools of thought, on both sides of the Atlantic Ocean, considered the progressive severe stenosis of

doi: 10.1111/j.1749-6632.2010.05784.x

atherosclerotic arteries as the final trigger for acute events.[7,8]

Many at that time believed that thrombosis was secondary to blood stasis following the infarction,[9] therefore a consequence and not a cause. Much of this knowledge was based on pathological sections of coronary arteries in autopsy material, where—retrospectively interpreted—the variable time lag between the onset of symptoms and death could account for the entire spectrum of possible findings, supporting both the causality and the noncausality hypotheses. If one study has to be quoted as a divider between the time of quarreling and the time of data consolidation indicating thrombosis as the final trigger of plaque instabilization, this is the study by De Wood *et al.*, which showed the greater incidence of total or subtotal coronary occlusion as an inverse function of time elapsed between symptom onset and the coronary angiography used to demonstrate the occlusion, with the retrieval of thrombotic material through a Fogarty catheter when such intervention was performed early in time.[10] Davies and coworkers, in 1985, illustrated the concept of plaque fissuring as a common cause of the acute manifestations of ischemic syndromes.[11] Closing the causality loop were, a few years later, the clinical trials demonstrating the possibility of dramatically reducing postmyocardial infarction mortality with antithrombotic treatments.[12–25] The focus at that point could shift upstream, to understand the precipitating causes of arterial thrombosis.

The inflammatory theory of atherosclerosis

Atherosclerotic lesions begin as fatty streaks, areas of intimal thickening due to the accumulation of "foam cells" (essentially lipid-laden macrophages), extracellular matrix, and lymphocytes.[26–30] Mature plaques, instead, consist of a thickened intima containing a lipid-rich core (*atheros*, gruel), surrounded by extracellular matrix. The lipid-rich core contains abundant tissue factor, capable of activating the extrinsic pathway of coagulation.[31,32] The presence of a collagen-rich cap and of smooth muscle cells around the core prevent the contact of blood with the thrombogenic component.[33] Smooth muscle cells, stimulated by the presence of transforming growth factor-β (TGF-β), fibroblast growth factor-2, (FGF2), and platelet-derived growth factor (PDGF) produced by cells participating in the inflammatory atherosclerotic process, indeed migrate from the media towards the intima, secreting several types of collagen and extracellular matrix (types I and II collagen, elastin, proteoglycans, fibronectin), contributing to the resistance to rupture of the fibrous cap and preventing thrombosis,[34–36] and switching from purely contractile cells into secreting and proliferating cells.[37] Matrix digestion instead occurs because of the production of specific proteinases, named matrix metalloproteinases (MMPs), mostly by activated macrophages arrived in the intima as transformation of circulating monocytes and their local replication.

Many such cellular aspects of atherosclerosis, such as the passage of monocytes from peripheral blood to tissues and macrophage activation, can be generically defined as "inflammatory."[32,38–40] Inflammation indeed, in its widest acception, can be defined as the passage of cellular elements from the blood to tissues in response to an injury on the vascular wall or of surrounding tissues.[41] Acute inflammation (with the prevailing involvement of neutrophils), cell-mediated immune responses (with a prevailing involvement of lymphocytes), and atherosclerosis (with the primary involvement of monocytes) are three forms of "inflammatory" reactions, all characterized by phenomena of passage of cells from the blood to the tissues. Inflammatory aspects are also the secretion of matrix and the collagenolytic degradation of the matrix by enzymes, mostly deriving from macrophages. Inflammation is therefore at the basis of all pathogenetic aspects of atherosclerosis, from lesion inception (the adhesion and migration of monocytes),[41,42] to their growth (accumulation of cells and matrix),[43] up to their clinical emergence (plaque fissuring).[44]

Many molecular aspects of vascular inflammation present in atherosclerosis are also common to acute inflammation and immune phenomena. All these have at their basis a condition of endothelial dysfunction (endothelial "activation") with the expression by the endothelium of an adhesive phenotype towards various leukocyte subtypes from peripheral blood.[41] The very same localization of atherosclerosis at particular sites of the vascular tree can be explained by such phenomena. Indeed, the preferential localization of atherosclerosis in branching sites, where shear stresses are low or oscillating, and produce temporal fluctuations in the tangential friction exerted on endothelial cells,[45–47] can be explained by the activation

of specific receptors responsive to such forces (shear stress response sensors) and the ensuing nuclear events involving shear stress response elements (SSRE). These produce a cytoskeletal rearrangements of endothelial cells, the surface expression of endothelial adhesion molecules (intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, etc.), and a reduction in the endothelial production of nitric oxide (NO) which, in contrast to the other molecules quoted above, increases in production when the shear stress is laminar.[48–50]

The specificity of atherosclerosis versus other forms of inflammation is essentially exerted at the endothelial level, where the early selective expression of adhesion molecules for monocytes allows these specific cells—and not neutrophils and lymphocytes—to adhere preferentially to the arterial endothelium, contrary to what occurs in other forms of inflammation. The immunoglobulin VCAM-1 is, in this context, very relevant in so far, through the interaction with the integrin very late activation antigen-4 (VLA4), expressed on monocytes and some lymphocyte classes.[51,52] VCAM-1-VLA4 binding determines the selective recruitment of this particular type of leukocytes. The expression of VCAM-1 by the endothelium is favored, besides by a high laminar shear stress and turbulent flow, by virtually all cardiovascular risk factors. As examples, stimuli to its expression are modified or oxidized LDL, advanced glycation endproducts found abundantly in diabetes and uremia, primary inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, as well as bacterial lipopolysaccharide (LPS).[53,54] These substances are therefore considered the soluble mediators of vascular atherosclerotic disease, which conjure with biomechanical factors in determining the extent and localization of atherosclerosis.

As a confirmation of the relationship of inflammatory phenomena with atherosclerosis, one may also remark the associations of atherosclerosis with classically "inflammatory" diseases. Rheumatoid arthritis, as an example, is associated with a high incidence of coronary heart disease. Both diseases show similarities in the activation of T-lymphocytes and mast cells, as well as in the production of TNF-α, IL-6, and adhesion molecules. The production of these and other cytokines by the synovial tissue in subjects with rheumatoid arthritis has similarities with the proatherogenic phenomena occurring in subjects with vascular disease, such as conditions of insulin resistance, dyslipidemias, endothelial activation, and a prothrombotic phenotype. Patients with rheumatoid arthritis feature high levels of C-reactive protein (CRP), a marker of inflammation associated with increased cardiovascular risk.[55]

The endothelium reacts to the proatherogenic activation with the production of counter-regulatory factors such as prostacyclin (PGI_2), NO, heparin-like complex polysaccharides able to activate antithrombin, type 1 plasminogen activator (PAI-1), protein S, thrombomodulin, and the secretion of ILs with antiproliferative and anti-inflammatory properties. In subjects with multiple risk factors for atherosclerosis, a simultaneous reduction in the endothelial production of such athero- and thrombo-protective mechanisms occurs.

The changes in the normal homeostatic balance between athero-protective and proatherogenic factors is generally explained by the activation, under the pressure of atherogenic risk factors, of a common proinflammatory mechanism, which in turns orchestrates the "pleiotropic" appearance of the endothelial proatherogenic phenotype. Such common mechanism may mostly be the transcription factor nuclear factor-κB (NF-κB) and a few other transcription factors. These represent the "central hub" of inflammation, a single intracellular system on which proinflammatory/proatherogenic stimuli converge determining its activation; and from which the various aspects of the pleiotropic endothelial proinflammatory phenotypes branch out (Fig. 1). Because of the multiplicity of the activation pathways (whereby the lack of one single activating stimulus may be compensated by the higher level of activation of other factors: the "redundancy" of the system), it is difficult to imagine that limiting the actions of one single risk factor may eradicate vascular disease, but it is logical to conceive that it can only serve to limit the problem. As a matter of fact, this intuitive approach to the choice of therapeutic targets in vascular disease has yielded appreciable results as the main current therapeutic strategies in vascular disease. One important consequence of this reasoning is also however that the central hub of inflammation (NF-κB, with the possible accessory contribution of other transcription factors, such as AP-1, GATA, egr-1), may be a reasonable target in vascular inflammation.[56]

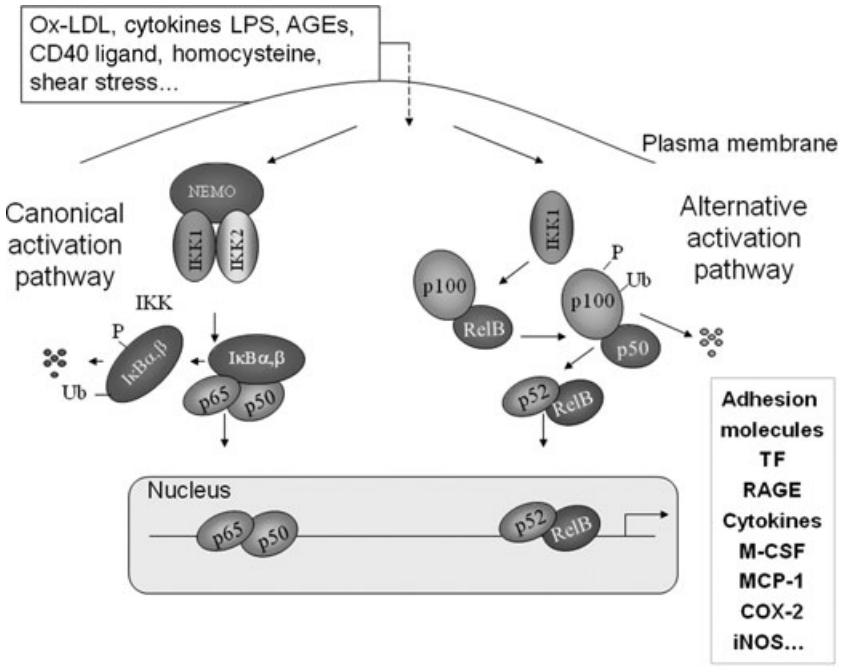

Figure 1. **Canonical and noncanonical pathways of activation of the transcription factor nuclear factor (NF)-κB. The NF-κB system comprises a series of heterodymeric molecules (monomers including Rel-A/p65, p50, p52, Rel-B, c-Rel) normally sequestered in the cytoplasm and bound to an inhibitor (I-κB). Under the influence of primary activating triggers (upper left inset), among which include inflammatory cytokines, modified LDL, etc., the increased generation of reactive oxygen species leads to the binding of I-κB with ubiquitin and the proteolytic degradation of I-κB. The heterodymers, made free from the inhibitor, can then migrate into the nucleus, where they bind specific recognition (consensus) sequences in the promoter region of a variety of genes of endothelial activation, with the final result of increased transcription of the respective genes (right lower inset). The partial overlap in properties of the primary triggers ("redundancy," e.g., the nearly identical pattern of endothelial activation evoked by IL-1 and TNF), results in a similar cellular activation by different stimuli. The fact that multiple genes, e.g., E-selectin, VCAM-1, and ICAM-1, are expressed even when a single cytokine activates the system epitomizes the "pleiotropy" of the system, another property of inflammatory cytokines. Reproduced, with changes, from De Caterina, R., A. Zampolli, G. Lazzerini & P. Libby. 2007. Endothelial activation and the initiation of atherosclerosis. In *Endothelial Dysfunctions in Vascular Disease*. De Caterina, R. & P. Libby, Eds.: 26–35. Blackwell-Futura. New York, with permission by the publisher.**

New biomarkers

This new knowledge on the central role of inflammation in atherogenesis has indeed yielded an entire new series of potential plasma biomarkers, different from those commonly used previously (e.g., based on the lipid profile), which largely overlap in reporting on pathogenetic mechanisms in vascular disease. Potential biomarkers here include VCAM-1, TNF-α, IL-1, IL-18, MMPs, CD40L, adiponectin, PAI-1, fibrinogen, and several others.[40] The ideal biomarker should be easily measured, have minimum circadian variations, and have a long half-life. CRP closely matches such a description.[57] Conversely, as an example, PAI-1 has a half-life of about 6 h and features important circadian variations.

CRP is predictive of cardiovascular events (myocardial infarction, stroke, and sudden cardiac death) in addition to measurements of LDL cholesterol.[58] For this reason, its measurement has been proposed in addition to the list of classical risk factors based on the Framingham algorithm.[59] Subjects with CRP < 1 mg/L are at low risk, whereas subjects with levels >3 mg/L are at high risk. Risk of ischemic heart disease and stroke in the latter situation indeed is increased by a factor of 1.3 and 1.6, respectively).[60] Conversely, the evidence that CRP may also be a mediator of vascular disease, and therefore itself a potential therapeutic target, appears much weaker, especially on the basis of recent Mendelian randomization studies.[60] Other new potential biomarkers stemming out of our recent

knowledge on vascular inflammation include CD40 ligand,[61] adiponectin (as an inverse correlate),[62] IL-18,[63] and some MMPs, potentially reporting on the risk of plaque rupture,[64] as well as TF, marking a prothrombotic phenotype.[65]

New therapeutic approaches

A hypothesis remains a hypothesis, no matter how attractive it is, until one can fully demonstrate its validity. The demonstration of the causality relationship for the inflammatory hypothesis of atherosclerosis requires the testing with adequate therapies. We have several therapies available with an impact on vascular atherosclerosis, but only few of them are practicable on a large scale in prevention strategies. These may largely be classified in three main groups:

1. therapies acting on primary atherogenic triggers,
2. therapies acting on the final effectors, and
3. therapies acting on the central common pathway of vascular inflammation.

We will briefly discuss these and highlight where novelties in this area may appear.

Therapies acting on primary atherogenic triggers

To combat inflammation linked to atherosclerosis, a first logical approach, followed for decades, has been to limit the classical risk factors of vascular diseases, with interventions on diet, physical activity, the cessation on smoking, or the effects on specific risk factors. Changes in lifestyles are unfortunately a constant source of frustration. The latest EuroAspire survey, which has produced a snapshot of practices of secondary prevention in eight European countries in 2005, comparing it to previous surveys of the same kind carried over in 1996 and 1998,[66] has shown how far we are from reaching the declared objectives. We are much better in prescribing drugs, mainly antihypertensive drugs and statins, than to induce people to stop smoking, reduce caloric intake or adopt healthy lifestyles. In any case, we are doing something good for atherosclerosis when we limit risk factors such as LDL cholesterol (with statins) or hypertension (with antihypertensive drugs).

In doing all this, we also reduce vascular inflammation. Statins are a particularly interesting class of drugs in this respect. Beyond reducing LDL cholesterol, statins also reduce markers of vascular inflammation, including IL-6, TNF, and CRP.[67,68] If a cholesterol-lowering therapy is anti-inflammatory, it should reduce CRP. This is exactly what happens: CRP is reduced by 15–50% with statins,[57,69–72] in what appears to be a class effect. If a reduced CRP really is a measure of the probability of cardiovascular complications, cardiovascular risk should parallel such a reduction. This is exactly what happens. A recent study has indeed reported that in patients with an acute coronary syndrome receiving statin therapy, subjects with CRP < 2 mg/dL had a reduced risk of reinfarction or vascular death compared with subjects with higher values (2.8 vs. 3.9 events per 100 persons-year; $P < 0.006$).[58] This benefit appears someway independent from reduction in LDL cholesterol. Therefore, the clinical benefit of statins seems to parallel anti-inflammatory effects. In any case, however, LDL reduction is itself an anti-inflammatory therapy, and this activity appears central to the beneficial effect of statins.

A similar reasoning can be done for drugs inhibiting the renin–angiotensin system, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers, commonly used throughout the spectrum of vascular disease.[73,74]

Therapies acting on the final effectors

Classical anti-inflammatory therapies are by many considered the essential ingredients of a therapeutic cocktail destined to demonstrate causality of inflammation in atherosclerosis. However, many classical anti-inflammatory therapies have contraindications in patients with vascular disease. Glucocorticoids, for example, were experimented with in the 1970s in the acute phase of a myocardial infarction, but their development with this indication was quickly halted owing to a higher incidence of heart rupture. Traditional nonsteroidal anti-inflammatory drugs, as well as more recently developed cyclooxygenase (COX)-2 selective inhibitors (coxibs), have a theoretical contraindication in that they limit vascular production of prostacyclin, which is mainly COX-2-dependent, and may compete for the antithrombotic effects of aspirin. Anticytokine drugs, which selectively hit one of the many final targets of inflammation, have been tested serendipitously. A therapy with etanercept, a derivative of a monoclonal antibody against TNF-α, has been experimented in a phase 3 study in heart failure (the RENEWAL study),

without appreciable results even limited to purely ischemic endpoints.[75] The MRC-ILA-HEART trial plans to study 186 patients with non-ST elevation myocardial infarction in the United Kingdom using anakinra, and IL-1 receptor antagonist (IL-1Ra). The study will evaluate efficacy and safety of the drug on circulating levels of CRP, as a prelude to a possible efficacy phase 3 study.[76] Similar approaches are the possible interferences with the production or activity of leukotrienes, with action at the level of 5-lipoxygenase or at leukotriene receptors. Although conceptually interesting, such approaches interfere with only some of the multiple mediators of inflammation potentially involved.

The case of dipyridamole

The total suppression of NF-κB activity, when tried in animal experiments, interferes with embryogenesis and with immune functions, and does not therefore appears as a viable option. On the contrary, first in *in vitro* and then *in vivo* in animal experiments, several drugs generically defined as "antioxidant" have been tested. Such drugs interfere with the generation of some reactive oxygen species, which are in turn activators of NF-κB. One such substances, succinobucol, has been brought to phase 3 in non-ST elevation acute coronary syndromes within the ARISE study, with mostly disappointing results.[77] One should however mention that the extent of inhibition of NF-κB with the *in vivo* administration of this drug was not as well documented as *in vitro*. Methotrexate, a drug commonly used in rheumatic diseases and relatively safe at low doses, might be an alternative worth testing, and this approach is currently being pursued.

An alternative option in this respect is to revisit old drugs for which some clinical efficacy has been already documented, and try to exploit novel notions about their mechanism of action. One such drug is dipyridamole. Originally branded as an antiplatelet agent, the drug has still an uncertain role in coronary heart disease (due to lack of data from well-performed clinical trials), but has evidence of efficacy in the prevention of ischemic stroke.[78] In our own hands (unpublished data), the drug reduces monocytoid cell and endothelial cell pro-MMP9 and MMP-9 after TNFα/phorbol myristate acetate or high-glucose stimulations, thus potentially reducing mechanisms of instabilization of atherosclerotic plaques (for the effect on monocytes, the main secretors of collagenolytic enzymes in the plaque, and for the effect on endothelial cells, where reduction on MMPs should lead to reduced plaque angiogenesis). Such effects appear to occur through inhibition of NF-κB activation. At the endothelial level, notably, dipyridamole induces endothelial cell COX-2 expression, alone or after TNFα/PMA stimulation, therefore not potentially curtailing the production of endothelial prostacyclin. These observations are intriguing, although their clinical relevance remains to be demonstrated.

Conclusions

Inflammation plays a central role in vascular disease, from plaque inception to plaque progression and instabilization. The now demonstrated sequence of events linking inflammation with vascular disease differs substantially from the old idea of vascular disease as a problem of lipid accumulation. Inflammatory markers such as CRP, derived from such knowledge, merit careful consideration to be included in our algorithms of risk evaluation. Knowledge of the mechanisms of vascular inflammation also holds the promise of unraveling new targets for therapeutics. Such new therapies may include the revisitation of the mechanism of action of old drugs.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Fry, D.L. 1968. Acute vascular endothelial changes associated with increased blood velocity gradients. *Circ. Res.* **22:** 165–197.
2. Fry, D.L. 1969. Certain histological and chemical responses of the vascular interface to acutely induced mechanical stress in the aorta of the dog. *Circ. Res.* **24:** 93–108.
3. Wright, H.P. 1971. Areas of mitosis in aortic endothelium of guinea-pigs. *J. Pathol.* **105:** 65–67.
4. Gofman, J.W. & F. Lindgren. 1950. The role of lipids and lipoproteins in atherosclerosis. *Science* **111:** 166–171.
5. Musliner, T.A., C. Giotas & R.M. Krauss. 1986. Presence of multiple subpopulations of lipoproteins of intermediate density in normal subjects. *Arteriosclerosis* **6:** 79–87.
6. Rudel, L.L., J.S. Parks, F.L. Johnson & J. Babiak. 1986. Low density lipoproteins in atherosclerosis. *J. Lipid Res.* **27:** 465–474.
7. Baroldi, G. 1965. Acute coronary occlusion as a cause of myocardial infarct and sudden coronary heart death. *Am. J. Cardiol.* **16:** 859–880.
8. Roberts, W.C. & L.M. Buja. 1972. The frequency and significance of coronary arterial thrombi and other observations

in fatal acute myocardial infarction: a study of 107 necropsy patients. *Am. J. Med*. **52:** 425–443.
9. Roberts, W. 1974. Editorial: coronary thrombosis and fatal myocardial ischemia. *Circulation* **49:** 1–3.
10. DeWood, M.A., J. Spores, R. Notske, *et al*. 1980. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N. Engl. J. Med*. **303:** 897–902.
11. Davies, M.J. & A.C. Thomas. 1985. Plaque fissuring—the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. *Br. Heart J*. **53:** 363–373.
12. Ganz, W., I. Geft, P.K. Shah, *et al*. 1984. Intravenous streptokinase in evolving acute myocardial infarction. *Am. J. Cardiol*. **53:** 1209–1216.
13. Lewis, H.D. Jr., J.W. Davis, D.G. Archibald, *et al*. 1983. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. Results of a Veterans Administration Cooperative Study. *N. Engl. J. Med*. **309:** 396–403.
14. Serruys, P.W., W. Wijns, M. Van Den Brand, *et al*. 1983. Is transluminal coronary angioplasty mandatory after successful thrombolysis? Quantitative coronary angiographic study. *Br. Heart J*. **50:** 257–265.
15. Spann, J.F., S. Sherry, B.A. Carabello, *et al*. 1982. High-dose, brief intravenous streptokinase early in acute myocardial infarction. *Am. Heart J*. **104:** 939–945.
16. Terrosu, P., G.V. Ibba, G.M. Contini & V. Franceschino. 1984. Angiographic features of the coronary arteries during intracoronary thrombolysis. *Br. Heart J*. **52:** 154–163.
17. Timmis, G.C., V. Gangadharan, A.M. Hauser, *et al*. 1982. Intracoronary streptokinase in clinical practice. *Am. Heart J*. **104:** 925–938.
18. Weinstein, J. 1982. Treatment of myocardial infarction with intracoronary streptokinase: efficacy and safety data from 209 United States cases in the Hoechst-Roussel registry. *Am. Heart J*. **104:** 894–898.
19. Baigent, C., R. Collins, P. Appleby, *et al*. 1998. ISIS-2: 10 year survival among patients with suspected acute myocardial infarction in randomised comparison of intravenous streptokinase, oral aspirin, both, or neither. The ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. *BMJ* **316:** 1337–1343.
20. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. 1988. Randomized trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. *J. Am. Coll. Cardiol*. **12:** 3A–13A.
21. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. 1988. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. *Lancet* **2:** 349–360.
22. Boissel, J.P. 1988. The ISIS-2 study (International Study of Infarction Survival). *Rev. Prat*. **38:** 1285–1288.
23. Fresco, C., M.G. Franzosi, A.P. Maggioni & G. Tognoni. 1990. The GISSI-2 trial: premises, results, epidemiological (and other) implications. *Clin. Cardiol*. **13:** VIII32–36.
24. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. 1990. GISSI-2: a factorial randomised trial of alteplase versus streptokinase and heparin versus no heparin among 12,490 patients with acute myocardial infarction. *Lancet* **336:** 65–71.
25. Hennekens, C.H. 1994. Thrombolytic therapy: pre- and post-GISSI-2, ISIS-3, and GUSTO-1. *Clin. Cardiol*. **17:** I15–I17.
26. Gerrity, R.G. 1981. The role of the monocyte in atherogenesis. II. Migration of foam cells from atherosclerotic lesions. *Am. J. Pathol*. **103:** 191–200.
27. Joris, I., T. Zand, J.J. Nunnari, *et al*. 1983. Studies on the pathogenesis of atherosclerosis. I. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am. J. Pathol*. **113:** 341–358.
28. Faggiotto, A., R. Ross & L. Harker. 1984. Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. *Arteriosclerosis* **4:** 323–340.
29. Stary, H.C. 1987. Macrophages, macrophage foam cells, and eccentric intimal thickening in the coronary arteries of young children. *Atherosclerosis* **64:** 91–108.
30. Rosenfeld, M.E., T. Tsukada, A.M. Gown & R. Ross. 1987. Fatty streak initiation in Watanabe Heritable Hyperlipemic and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis* **7:** 9–23.
31. Clinton, S.K., R. Underwood, L. Hayes, *et al*. 1992. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. *Am. J. Pathol*. **140:** 301–316.
32. Libby, P. & P. Theroux. 2005. Pathophysiology of coronary artery disease. *Circulation* **111:** 3481–3488.
33. Annex, B.H., S.M. Denning, K.M. Channon, *et al*. 1995. Differential expression of tissue factor protein in directional atherectomy specimens from patients with stable and unstable coronary syndromes. *Circulation* **91:** 619–622.
34. Geng, Y.J., Q. Wu, M. Muszynski, *et al*. 1996. Apoptosis of vascular smooth muscle cells induced by in vitro stimulation with interferon-gamma, tumor necrosis factor-alpha, and interleukin-1 beta. *Arterioscler. Thromb. Vasc. Biol*. **16:** 19–27.
35. Thyberg, J. 1996. Differentiated properties and proliferation of arterial smooth muscle cells in culture. *Int. Rev. Cytol*. **169:** 183–265.
36. Newby, A.C. 1997. Molecular and cell biology of native coronary and vein-graft atherosclerosis: regulation of plaque stability and vessel-wall remodelling by growth factors and cell-extracellular matrix interactions. *Coron. Artery Dis*. **8:** 213–224.
37. Peoples, G.E., S. Blotnick, K. Takahashi, *et al*. 1995. T lymphocytes that infiltrate tumors and atherosclerotic plaques produce heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor: a potential pathologic role. *Proc. Natl. Acad. Sci. USA* **92:** 6547–6551.
38. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med*. **340:** 115–126.
39. Hansson, G.K., P. Libby, U. Schonbeck & Z.Q. Yan. 2002. Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ. Res*. **91:** 281–291.
40. Packard, R.R. & P. Libby. 2008. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin. Chem*. **54:** 24–38.

41. De Caterina, R., M. Massaro & P. Libby. 2007. Endothelial functions and dysfunctions. In *Endothelial Dysfunctions in Vascular Disease*. De Caterina, R. & P. Libby, Eds.: 3–25. Blackwell-Futura. New York.
42. De Caterina, R. 1999. Inflammation and early events in atherogenesis. *Cardiologia* **44**(Suppl 1): 109–115.
43. De Caterina, R., A. Zampolli, G. Lazzerini & P. Libby. 2007. Endothelial activation and the initiation of atherosclerosis. In *Endothelial Dysfunctions in Vascular Disease*. De Caterina, R. & P. Libby, Eds.: 26–35. Blackwell-Futura. New York.
44. De Caterina, R., A. Zampolli, S. Del Turco & P. Libby. 2007. Mechanisms of plaque progression and complications. In *Endothelial Dysfunctions in Vascular Disease*. De Caterina, R. & P. Libby, Eds.: 36–54. Blackwell-Futura. New York.
45. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. *Arteriosclerosis* 1985; **5:** 293–302.
46. Gimbrone, M.A., Jr., T. Nagel, J.N. Topper. 1997. Biomechanical activation: an emerging paradigm in endothelial adhesion biology. *J. Clin. Invest*. **99:** 1809–1813.
47. Davies, P.F. 1995. Flow-mediated endothelial mechanotransduction. *Physiol. Rev*. **75:** 519–560.
48. Ando, J., H. Tsuboi, R. Korenaga, *et al*. 1994. Shear stress inhibits adhesion of cultured mouse endothelial cells to lymphocytes by downregulating VCAM-1 expression. *Am. J. Physiol.* **267:** C679–C687.
49. De Caterina R, Libby P, Peng HB, *et al*. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J. Clin. Invest.* 1995; **96:** 60–68.
50. Khan, B.V., D.G. Harrison, M.T. Olbrych, *et al*. 1996. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc. Natl. Acad. Sci. USA* **93:** 9114–9119.
51. Bochner, B.S., F.W. Luscinskas, M.A. Jr. Gimbrone, et al. 1991. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J. Exp. Med*. **173:** 1553–1557.
52. Cybulsky, M.I., J.W. Fries, A.J. Williams, *et al*. 1991. Alternative splicing of human VCAM-1 in activated vascular endothelium. *Am. J. Pathol*. **138:** 815–820.
53. Nagel, T., N. Resnick, W.J. Atkinson, C.F. Jr. Dewey & M.A., Jr. Gimbrone. 1994. Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J. Clin. Invest*. **94:** 885–891.
54. Resnick, N., T. Collins, W. Atkinson, *et al*. 1993. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive element. *Proc. Natl. Acad. Sci. USA* **90:** 4591–4595.
55. Libby, P. 2008. Role of inflammation in atherosclerosis associated with rheumatoid arthritis. *Am. .J Med*. **121:** S21–S31.
56. Collins, T. 1993. Endothelial nuclear factor-kB and the initiation of the atherosclerotic lesion. *Lab. Invest*. **68:** 499–508.
57. Ridker, P.M., N. Rifai, M. Clearfield, *et al*. 2001. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N. Engl. J. Med*. **344:** 1959–1965.
58. Ridker, P.M., C.P. Cannon, D. Morrow, *et al*. 2005. C-reactive protein levels and outcomes after statin therapy. *N. Engl. J. Med*. **352:** 20–28.
59. Koenig, W., H. Lowel, J. Baumert & C. Meisinger. 2004. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. *Circulation* **109:** 1349–1353.
60. Zacho, J., A. Tybjaerg-Hansen, J.S. Jensen, *et al*. 2008. Genetically elevated C-reactive protein and ischemic vascular disease. *N. Engl. J. Med*. **359:** 1897–1908.
61. Schonbeck, U. & P. Libby. 2001. CD40 signaling and plaque instability. *Circ. Res*. **89:** 1092–1103.
62. Patel, D.A., S.R. Srinivasan, J.H. Xu, *et al*. 2006. Adiponectin and its correlates of cardiovascular risk in young adults: the Bogalusa Heart Study. *Metabolism* **55:** 1551–1557.
63. Skurk, T., H. Kolb, S. Muller-Scholze, *et al*. 2005. The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. *Eur. J. Endocrinol*. **152:** 863–868.
64. Inokubo, Y., H. Hanada, H. Ishizaka, *et al*. 2001. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. *Am. Heart J*. **141:** 211–217.
65. Mallat, Z. & A. Tedgui. 2001. Current perspective on the role of apoptosis in atherothrombotic disease. *Circ. Res*. **88:** 998–1003.
66. EUROASPIRE I and II Group. European Action on Secondary Prevention by Intervention to Reduce Events. 2001. Clinical reality of coronary prevention guidelines: a comparison of EUROASPIRE I and II in nine countries. *Lancet* **357:** 995–1001.
67. Ridker, P.M., N. Rifai, M. Pfeffer, *et al*. 2000. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation* **101:** 2149–2153.
68. Ridker, P.M., N. Rifai, M.J. Stampfer & C.H. Hennekens. 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* **101:** 1767–1772.
69. Albert, M.A., E. Danielson, N. Rifai & P.M. Ridker. 2001. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA* **286:** 64–70.
70. Wissen, S., M.D. Trip, T.J. Smilde, *et al*. 2002. Differential hs-CRP reduction in patients with familial hypercholesterolemia treated with aggressive or conventional statin therapy. *Atherosclerosis* **165:** 361–366.
71. Ree, M.A., M.V. Huisman, H.M. Princen, *et al*. 2003. Strong decrease of high sensitivity C-reactive protein with high-dose atorvastatin in patients with type 2 diabetes mellitus. *Atherosclerosis* **166:** 129–135.
72. Nissen, S.E., E.M. Tuzcu, P. Schoenhagen, *et al*. 2004. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* **291:** 1071–1080.

73. De Caterina R. & C. Manes. Inflammation in early atherogenesis: impact of ACE inhibition. *Eur. Heart J*. 2003:A15–A24.
74. Graham, I., D. Atar, K. Borch-Johnsen, *et al*. 2007. European guidelines on cardiovascular disease prevention in clinical practice: executive summary. *Eur. Heart J*. **28:** 2375–2414.
75. Mann, D.L., J.J. McMurray, M. Packer, *et al*. 2004. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation* **109:** 1594–1602.
76. Crossman, D.C., A.C. Morton, J.P. Gunn, *et al*. 2008. Investigation of the effect of Interleukin-1 receptor antagonist (IL-1ra) on markers of inflammation in non-ST elevation acute coronary syndromes (The MRC-ILA-HEART Study). *Trials* **9:** 8.
77. Tardif, J.C., J.J. McMurray, E. Klug, *et al*. 2008. Effects of succinobucol (AGI-1067) after an acute coronary syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet* **371:** 1761–1768.
78. Albers, G.W., P. Amarenco, J.D. Easton, *et al*. 2008. Antithrombotic and thrombolytic therapy for ischemic stroke: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* **133:** 630S–669S.