The Role of Inflammation in Plaque Disruption and Thrombosis

Kuang-Yuh Chyu, MD, Prediman K. Shah, MD
Division of Cardiology, Cedars-Sinai Medical Center, Los Angeles, CA

Most of the serious clinical manifestations (such as unstable angina, acute MI, and many cases of sudden death) of coronary atherosclerosis result from thrombosis, usually occurring on a disrupted atherosclerotic plaque. Plaques prone to disruption have large lipid-rich cores with evidence of cap-thinning and active inflammation. Inflammatory cells may contribute to both plaque disruption and subsequent thrombosis. Here we review the evidence for the involvement of inflammation in plaque disruption and thrombosis and the potential clinical implications of this pathophysiologic paradigm.
[Rev Cardiovasc Med. *2001;2(2):82–91*]

Key words: Atherosclerosis • Coronary artery disease • Inflammation • Thrombosis

Coronary atherosclerotic disease is the leading cause of death in the United States and in many developing countries. The clinical spectrum of coronary atherosclerosis varies from an asymptomatic state and stable angina to acute coronary syndromes, such as unstable angina, acute myocardial infarction, and sudden cardiac death. The progression of disease is often unpredictable, with the natural history punctuated by rapid worsening of coronary luminal obstruction causing sudden development of total or near-total occlusion. This rapid change is most frequntly due to thrombus formation on top of a disrupted plaque; however, abnormal vasomotor tone may also play a contributory role in certain instances.

The Concept of "Vulnerable Plaques"

Histopathologic studies have demonstrated certain characteristic features in ruptured plaques, and by inference these attributes have been suggested to be determinants of vulnerability to disruption. These features include[1-9]:

1. A large, predominantly acellular, soft, lipid-rich core
2. A thin fibrous cap with focal areas of thinning
3. Inflammatory cell infiltration in the plaque and fibrous caps, especially at shoulder regions and underneath sites of rupture and in the adventitia
4. Reduced smooth muscle cell density along with reduced collagen content in the fibrous caps
5. Increased neovascularity in the plaque
6. Outward or positive remodeling of vessel size.

Since outward remodeling can preserve the lumen despite a large amount of plaque in the wall, remodeled vessels may not demonstrate significant angiographically detectable stenosis and may thus remain undetected until disruption occurs. A high frequency of positive or outward remodeling in vulnerable and disrupted plaques may explain, in part, the observation that 50% to 70% of acute coronary syndromes arise from plaques that are mildly or moderately obstructive prior to disruption.

In comparison with intact plaques, disrupted plaques contain a substantially greater number of inflammatory cells, often in the fibrous cap and around the lipid core with a preferential concentration at rupture-prone shoulders of the plaque or underneath the thinned-out or disrupted fibrous cap.[2,3,5,6] In addition, adventitial inflammation is common in lesions responsible for acute myocardial infarction. Monocyte-derived macrophages, often bearing markers of activation, are the most abundant component of the inflammatory response, but activated T lymphocytes and degranulating mast cells are also found in larger numbers in disrupted plaques than in intact plaques.[3,5,6] Plaque fissure or rupture is likely to result from loss of collagen matrix in the fibrous cap, leading to thinning, weakening, and eventual rupture. Several studies have recently demonstrated that macrophages and, to a lesser extent, smooth-muscle-cell-derived foam cells in atherosclerotic plaques produce a family of matrix-degrading metalloproteinases (MMPs) and cysteine proteases (cathepsin S) that are capable of degrading virtually all components of the extracellular matrix.[10–12] Similarly, reduced smooth muscle content (from programmed cell death, or apoptosis) and reduced synthetic function (from T-cell-derived cytokines) may contribute to reduced capacity for collagen synthesis, which in turn contributes to matrix dysregulation and plaque disruption. Increased apoptosis involving smooth muscle cells in atherosclerosis has been described and attributed to cytotoxic effects of macrophage-derived products (reactive oxygen species, epidermal growth factor [EGF]-like domain of tenascin-C), oxidized low-density lipoprotein (oxLDL) and other mediators.

Disruption of atherosclerotic plaque and subsequent thrombosis is a key precipitant of potentially lethal acute coronary syndromes.

Plaque Erosion

In a subset of patients who die suddenly from acute coronary syndromes, the anatomic substrate for coronary thrombosis is superficial endothelial erosion of a proteoglycan-rich atherosclerotic lesion without rupture of the fibrous cap. Plaque erosion is characterized by less calcification, less luminal narrowing, and less inflammatory cell infiltration than is seen in ruptured plaques.[13] Patients who die of plaque erosion tend to be younger, to be female, to smoke cigarettes, and to have lower total/high-density-lipoprotein (HDL) cholesterol ratios than patients with plaque disruption.[13–15] Although the precise reason for erosion and subsequent thrombosis is unclear, it is possible that superficial endothelial erosion may occur from loss of basement membrane anchoring due to the basement-membrane-degrading activity of certain MMPs by cells within the vessel wall. Furthermore, a prominent circulating prothrombotic state created by cigarette smoking and/or by estrogens in females may create a suitable milieu for thrombosis at sites of superficial arterial injury.

Thrombosis Following Plaque Rupture

Following plaque disruption, coronary thrombosis may supervene, depending on the thrombogenicity of plaque components exposed, the severity of local stenosis with its effect on shear stress and shear-induced platelet activation, and the prevailing systemic thrombotic-thrombolytic balance. The main thrombogenic components of the plaque include the collagen and the lipid core. The high thrombogenicity of the lipid core may be due to its high content of catalytically active tissue factor, produced predominantly by macrophages in the atherosclerotic plaque.[16,17] Tissue factor, on exposure to circulating blood, interacts with factor VIIa, and this complex in turn activates factor X. Activated factor X initiates the cascade by cleaving prothrombin to thrombin, which in turn triggers coagulation with fibrin deposition and platelet activation, resulting in thrombus formation. We have recently demonstrated that phenotypic differentiation of monocytes and macrophages as well as exposure of macrophages to oxLDL cholesterol markedly enhances their tissue factor content and procoagulant activity.[18] Recent studies have also shown that activated circulating leukocytes

(mostly monocytes but probably neutrophils as well) can transfer tissue-factor-rich microparticles to platelets, which make them capable of triggering thrombosis. This transfer involves the interaction of CD15 and tissue factor with platelets.[19,20] These findings may thus explain the presence of tissue factor in platelet-rich thrombi deposited on denuded arterial wall even when the wall has no stainable tissue factor. These observations may have relevance to thrombosis associated with plaque erosion.[13–15]

Extrinsic factors such as local flow disturbance related to severity of local stenosis, altered local geometry leading to shear-induced platelet activation, and the systemic thrombotic-thrombolytic balance affect the thrombotic response following plaque disruption. Elevated levels of fibrinogen, increased factor VII–mediated procoagulant activity, enhanced platelet aggregability, and depressed endogenous fibrinolysis have all been shown to have a relationship with risk of atherothrombotic vascular events.

Endothelial dysfunction may also contribute to the thrombotic consequences of plaque rupture. Normal vascular endothelium plays a critical role in the regulation of vascular tone by releasing vasodilators (eg, nitric oxide, prostacyclin) and vasoconstrictors (eg, endothelin) and in maintenance of a balance between thrombosis and thrombolysis by releasing antithrombotic (eg, nitric oxide, protein C, heparin sulfate, ecto-adenosine diphosphate [ADP]ase), prothrombotic (eg, tissue factor, endothelin), profibrinolytic (eg, tissue plasminogen activator), and antifibrinolytic (eg, plasminogen activator inhibitor-1) agents. The endothelial dysfunction associated with atherosclerosis and the presence of risk factors for atherosclerosis increase the potential for excessive and paradoxic vasoconstriction, the expression of adhesion molecules that recruit monocytes and other inflammatory cells into the arterial wall, and the promotion of a prothrombotic and antifibrinolytic state.[21–23] Therefore, abnormal endothelial function may also play a role in thrombosis following plaque disruption.

A high frequency of positive or outward remodeling in vulnerable and disrupted plaques may explain, in part, the observation that 50% to 70% of acute coronary syndromes arise from plaques that are mildly or moderately obstructive prior to disruption.

Potential Triggers of Plaque Inflammation and Therapeutic Approaches

Histologic evidence of accumulation of activated inflammatory cells (macrophages, T lymphocytes, mast cells) in atherosclerotic plaques, especially at sites of plaque disruption or impending plaque disruption, coupled with the increased circulating levels of inflammatory markers (C-reactive protein, serum amyloid A, leukocyte adhesion molecules) seen in atherosclerotic disease has suggested a potential link between inflammation and atherothrombosis.[24–27] This view is further supported by epidemiologic studies demonstrating adverse prognostic implications of elevated levels of circulating inflammatory markers as well as by the plausible pathophysiologic underpinnings of plaque disruption and thrombosis discussed previously.

The precise triggers for inflammation in atherosclerosis remain unknown, but dyslipidemia (increased LDL cholesterol and triglyceride and lowered HDL cholesterol), hypertension, physical and mental stress, diabetes mellitus, estrogen deficiency, increased angiotensin II levels, cigarette smoking, hyperhomocysteinemia, infection, and immune modulation have all been implicated. Different therapeutic strategies against these triggers have been proposed and tested experimentally and/or clinically. Among them, cholesterol lowering, angiotensin-converting enzyme inhibition, smoking cessation, and diabetes control have been clinically proven to be effective in reducing the morbidity and mortality of coronary artery disease, most likely through plaque stabilization.

Concept of plaque stabilization. Reduced risk for plaque disruption and thrombosis may be achieved through change in lipid content, inflammatory cell infiltration, and activity in the plaque, leading to plaque stabilization. In hypercholesterolemic rabbits, lipid lowering reduces the expression and activity of MMP and tissue factor and promotes accumulation of mature smooth muscle cells and collagen, all of which favor plaque stabilization and reduction of thrombotic complications.[38–40] Such a plaque stabilization effect was recently further demonstrated by our group in a clinical lipid-lowering study, which showed that pravastatin treatment for 3 months increased collagen content and decreased lipid content, inflammation, and matrix metalloproteinase expression in human carotid plaques.[41] Thus, lipid lowering may work by stabilizing vulnerable plaques, preventing disruption and/or subsequent thrombosis. This therapeutic paradigm

opens up a new approach to reducing the adverse consequences of atherosclerosis.

Dyslipidemia. Hypercholesterolemia is associated with increased risk for CAD, as demonstrated in numerous observational and epidemiologic studies. Hypercholesterolemia leading to increased production of oxLDL cholesterol can cause endothelial dysfunction; stimulates expression of various MMPs in endothelium, vascular smooth muscle cells, and monocyte-derived macrophages;[28,29] and enhances tissue factor expression and activity.[30,31] All of these effects have been implicated in inflammation, plaque formation, plaque disruption, and subsequent thrombosis. Randomized, placebo-controlled trials have clearly demonstrated that LDL or triglyceride-lowering therapy (with increases in HDL) can reduce the morbidity and mortality of CAD in primary and secondary prevention.[32–36]

A number of serial angiographic trials evaluating the efficacy of lipid lowering and lifestyle modification have demonstrated a disproportionately greater reduction in the incidence of atherothrombotic clinical events (ie, acute coronary syndromes and strokes) compared with relatively trivial change in the severity of coronary stenosis.[37] This clinical-angiographic paradox led to the concept that risk factor modification, especially lipid lowering, may reduce the incidence of plaque disruption or thrombosis, and thus the number of clinical events, by changing the biology of the plaque rather than its overall size, volume, or the severity of stenosis.[37]

The strong negative relationship between HDL cholesterol levels and CAD in epidemiologic studies suggested that HDL-based therapy could be a new therapeutic paradigm for atherosclerotic vascular disease.[42] The antiatherogenic effects of HDL have been largely attributed to its apo A-I component. Thus, overexpression of human apo A-I transgene in mice results in elevated apo A-I and HDL levels, with substantial protection against dyslipidemia.[43,44] Systemic gene therapy delivering the apo A-I gene in experimental animals results in similar results and regression of atherosclerosis.[45,46] Our group further demonstrated that intravenous administration of reconstituted or recombinant apo A-I-HDL reduced plaque inflammation and lipid content, prevented progression of atherosclerotic lesions,[47] and also provided beneficial vascular protective effects.[48,49]

Atherosclerosis is a chronic inflammatory disease associated with activation of the immune system.

Infection. A growing body of evidence has suggested that infection may contribute to atherosclerosis and/or its destabilization. A number of infectious agents have been implicated, including *Chlamydia pneumoniae, Helicobacter pylori*, herpes simplex virus, and cytomegalovirus.[50] These infectious agents and their secreted products can directly tilt the balance between prothrombotic and fibrinolytic activity toward a more hypercoagulable state in endothelium, enhance leukocyte adhesion to endothelium, and promote the production of proinflammatory cytokines.[50] Indirectly, these infectious agents may also elicit a systemic inflammatory response that enhances proinflammatory cytokine gene expression in local atheroma.[50] Furthermore, infectious agents may activate the immune system, leading to immune-mediated vascular damage through molecular or antigenic mimicry.[51]

Although direct evidence linking infectious agents to atherosclerosis in humans is lacking, animal models have suggested that infection can accelerate atherosclerosis in the presence of hyperlipidemia.[52–54] It has been observed that only hypercholesterolemic mice, not wild-type mice, develop atherosclerotic lesions when infected with Herpesvirus.[54] A similar synergistic effect of hypercholesterolemia and infection in atherosclerotic lesion formation was observed in *Chlamydia*-infected LDL-receptor-deficient mice.[55] Wild-type mice raised in a pathogen-free environment developed more aortic lesions than wild-type mice raised in conventional conditions, whereas IL-10 knockout mice raised in conventional conditions developed larger aortic lesions than IL-10 knockout mice raised in a pathogen-free environment.[56] This suggests that infection alone may not elicit an atherogenic response and that other factors (such as cholesterol level or cytokine balance) may be necessary to facilitate or modulate atherogenesis.

It is not clear whether antichlamydial therapy stabilizes vulnerable plaques by modifying plaque composition; nevertheless, clinical trials have been conducted to explore the hypothesis that antichlamydial treatment with macrolides would reduce the risk of coronary artery disease (CAD). Two small pilot studies have suggested that macrolide therapy may reduce the risk of recurrent acute coronary syndromes in patients with CAD.[57,58] However, these findings were not confirmed in

a somewhat larger, randomized secondary prevention trial.[59] Additional large-scale trials are under way to test the hypothesis that antichlamydial antibiotics reduce CAD events.

Homocysteine. Severe hyperhomocysteinemia due to congenital deficiency of cystathionine β-synthase or N^5,N^{10}-methylene-tetrahydrofolate reductase (MTHFR) is rare but has long been recognized to cause premature atherosclerotic vascular disease. Recently, mild hyper-homocysteinemia was identified as an independent risk factor for atherosclerosis in the coronary, cerebral, and peripheral vasculature[60,61] and its levels were found to predict coronary mortality in patients with established coronary disease.[62] Multiple factors, including age, sex, smoking status, genetics, nutritional status, disease state, and drug therapy, may influence plasma homocysteine levels. A detailed description of individual factors is beyond the scope of this article and can be found in the literature.[63–65] A mutant MTHFR allele due to a single-base-pair change from cytosine to thymine at nucleotide 677 of the MTHFR gene was once hypothesized to be a genetic risk factor for premature cardiovascular disease, based on the association between mutation homozygosity and higher homocysteine level and on the high prevalence of this MTHFR mutation among the American, Canadian, and Dutch populations.[66–68] However, subsequent studies did not confirm this hypothesis.[69–71]

The exact cellular mechanism(s) responsible for the role of homocysteine in vascular injury is unknown. However, experimental evidence suggests homocysteine may act on various cellular components that are responsible for plaque rupture and atherothrombosis. Homocysteine causes an inflammatory response in endothelium, as evidenced by increased P-selectin and intercellular adhesion molecule-1 (ICAM-1) expression, which in turn increases leukocyte rolling, adherence, and transmigration across vessel walls.[72] Homocysteine can directly activate MMP-2,[73] which may contribute to extracellular matrix degradation, leading to plaque rupture. A hypercoagulable state can also be induced by homocysteine, since homocysteine enhances tissue factor activity in endothelium and monocytes; promotes platelet aggregation and thromboxane biosynthesis; and, in conjunction with oxLDL cholesterol, increases GP IIb/IIIa/fibrinogen-dependent platelet adhesion to endothelium.[74–77]

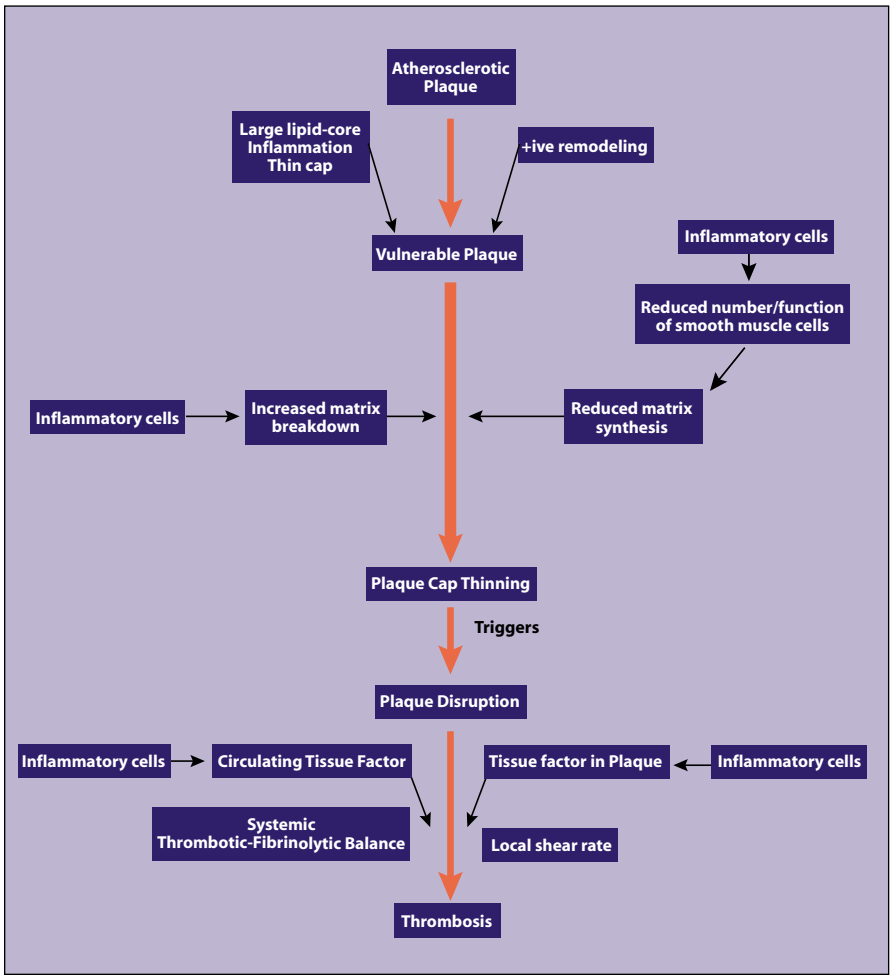

Figure 1. *Schematic describing the pathophysiology of plaque rupture and thrombosis.*

Folic acid and vitamin B6 supplementation lowers plasma homocysteine levels and improves markers of endothelial dysfunction.[78] It also induces regression of carotid plaques, as assessed by 2-dimensional B-mode ultrasound measurement.[79] A recent randomized, placebo-controlled trial showed that homocysteine-lowering treatment in healthy siblings of patients with premature atherothrombotic disease is associated with decreased occurrence of

abnormal exercise electrocardiography results.[80] Although either clinically irrelevant or soft endpoints were used in these studies, their data seemed to suggest a beneficial role of homocysteine-lowering therapy. Currently, there are no data from randomized trials using clinically relevant hard endpoints to substantiate the beneficial effect of homocysteine lowering by vitamin supplementation.

Hypercholesterolemia is associated with increased risk for CAD.

Angiotensin II and angiotensin-converting enzyme inhibitors. Activation of the renin-angiotensin-aldosterone system increases the risk of cardiovascular events. This may be due to the fact that angiotensin II can stimulate growth factors that are important in atherogenesis, activate an inflammatory response in vascular smooth muscle cells, promote smooth muscle cell hypertrophy, oxidize LDL-cholesterol particles, increase oxidative stress and superoxide production, and activate nuclear factor-κB (NF-κB) nuclear transcription, leading to expression of endothelial vascular cell adhesion molecule (VCAM)-1.[81–85] In experimental animals, angiotensin II promotes atherosclerotic lesions and aneurysm formation,[86] and treatment with angiotensin converting enzyme inhibitors (ACEIs) reduces the incidence of atherosclerotic lesions.[87–89] This atherosclerosis reduction effect could translate to a clinically relevant cardiac event reduction effect, since a recent randomized trial showed that ramipril treatment reduces the rates of death, myocardial infarction, and stroke in patients with high risk factors for atherosclerotic vascular disease.[90]

Immune modulation. Atherosclerosis is a chronic inflammatory disease associated with activation of the immune system. Atherosclerotic plaques contain activated macrophages expressing major histocompatibility complex (MHC) II molecules that allow them to present antigens to T lymphocytes. Activated T lymphocytes secrete proinflammatory cytokines such as interferon gamma and tumor necrosis factor alpha that further promote inflammatory responses. The nature of the antigens that can be processed by activated macrophages is unclear, but heat shock protein and oxLDL are possible candidates. Heat shock protein has been shown to activate vascular endothelium, smooth muscle cells, and macrophages[91] and antibody against heat shock protein has also been associated with seropositivity to *Chlamydia pneumoniae* and *Helicobacter pylori* in patients with atherosclerosis.[51] Autoantibodies against oxLDL exist in animals and humans[92–94]; however, their role in atherogenesis and whether their level predicts the severity of atherosclerosis remain controversial. Patients with atherosclerotic vascular disease have been shown to have higher levels of autoantibodies against oxLDL.[95–100] However, observations that oxLDL autoantibodies do not predict atherosclerotic vascular disease[101,102] or even protect against atherosclerosis[103] have also been reported.

Though the exact role of oxLDL antibody in atherogenesis remains unclear, its existence in vivo may provide the basis for immune modulatory therapy. In experimental animals, intravenous immunoglobulin treatment reduced atherosclerosis by modulating T-cell activity and/or antibody production.[104] Immunizing hyperlipidemic animals with homol-

Main Points

- Thrombosis causes most of the acute coronary events associated with atherosclerosis.
- Disrupted plaques contain more inflammatory cells than intact plaques.
- Increased fibrinogen levels, procoagulant activity, and platelet aggregability and depressed endogenous fibrinolysis increase the risk of atherothrombosis.
- Either rupture or erosion of atherosclerotic plaques can cause sudden death from acute coronary syndromes.
- The collagen and the lipid core in plaques are highly thrombogenic.
- Risk factor modification may work by stabilizing the plaque rather than changing its size or the severity of stenosis.
- Infection, homocysteine, angiotensin II, and immune factors may influence the risk of atherothrombosis and may be appropriate targets for intervention.

ogous oxLDL decreased atherosclerotic lesion size, with concomitant development of antibody against oxLDL.[105–107] Of interest is the observation that immunizing animals with homologous native LDL achieved similar plaque-reducing effects[105,107] without formation of antibodies against oxidation-specific epitopes.[107] This plaque-reducing effect of immune modulation therapies appears to be independent of cholesterol lowering, because plasma cholesterol levels were either not changed[104–106] or only mildly reduced with therapies.[107]

After antigen activation, T lymphocytes secrete a variety of cytokines that can modulate atherogenesis. Activated T cells can be induced to develop along 2 functional subsets: Th1 or Th2, based on their secreted cytokine profiles. Interferon gamma (IFN-gamma) and interleukin-12 (IL-12) are typical Th1 cytokines, and both promote atherosclerosis.[108,109] Typical Th2 cytokines include interleukin-4 (IL-4) and interleukin-10 (IL-10). IL-10 deactivates macrophages and T cells, inhibits NF-κB activation,[110] and reduces MMP and tissue factor expression,[111,112] which have all been attributed to the anti-inflammatory properties of IL-10. Since Th1 and Th2 cytokines cross-regulate each other, it is possible that Th1/Th2 balance in vivo may influence atherogenesis. This concept has been tested in experimental animals. Mice lacking Th1 signaling (INF-gamma receptor knockout) or having enhanced Th2 signaling (IL-10 transgenic mice) developed smaller atherosclerotic lesions,[108,113] less cellular infiltration in lesions, and higher collagen content in plaques,[108] implying a more stable plaque phenotype. On the other hand, mice lacking Th2 signaling (IL-10 knockout mice) developed more atherosclerotic lesions, higher numbers of infiltrating T cells, and lower collagen content in plaques.[56]

Although immune modulatory therapy and Th1/Th2 cytokine manipulation strategy appears promising in reducing atherosclerosis, this approach is still too new for clinical testing or exploration. Whether these therapeutic modalities will reduce plaque disruption and subsequent thrombosis is also unknown.

Conclusion

Considerable data from in vitro and in vivo studies of vascular biology, together with indirect evidence from clinical trials of lipid-lowering, lifestyle-modification, or risk factor–modifying interventions, provide strong support for the concept that disruption of atherosclerotic plaque and subsequent thrombosis is a key precipitant of potentially lethal acute coronary syndromes. Certain characteristics of plaques, including the size and composition of the lipid core, the structure and composition of the fibrous cap, and the presence of a local inflammatory process, predispose the plaque to disruption. Therefore, interventions aimed at decreasing plaque vulnerability and promoting plaque stabilization may reduce the risk of acute coronary syndromes. Several possible therapeutic approaches to reduce inflammatory responses of atherosclerosis are under study. Although not yet rigorously validated in humans, except for cholesterol reduction, plaque-stabilizing therapies may prove to be an important clinical strategy for preventing the lethal consequences of coronary atherosclerosis and other forms of atherothrombotic vascular disease. ■

References

1. Burleigh MC, Briggs AD, Lendon CL, et al. Collagen type I and III, collagen content, GAGs and mechanical strength of human atherosclerotic plaque caps: span-wise variations. *Atherosclerosis*. 1992;96:71–81.
2. Davies MJ, Richardson PD, Woolf N, et al. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J*. 1993;69:377–381.
3. Kovanen PT, Kaartinen M, Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation*. 1995;92:1084–1088.
4. Lendon CL, Davies MJ, Born GVR, et al. Atherosclerotic plaque caps are locally weakened when macrophage density is increased. *Atherosclerosis*. 1991;87:87–90.
5. Moreno PR, Falk E, Palacios IF, et al. Macrophage infiltration in acute coronary syndromes: implications for plaque rupture. Circulation. 1994;90:775–778.
6. Van der Wal AC, Becker AE, van der Loos CM, et al. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89:36–44.
7. Laine P, Naukkarinen A, Heikkila L, Penttila A, Kovanen PT. Adventitial mast cells connect with sensory nerve fibers in atherosclerotic coronary arteries. *Circulation*. 2000;101:1665–1669.
8. Pasterkamp G, Schoneveld AH, van der Wal AC, et al. Relation of arterial geometry to luminal narrowing and histologic markers for plaque vulnerability: the remodeling paradox. *J Am Coll Cardiol*. 1998;32:655–662.
9. Schoenhagen P, Ziada KM, Kapadia SR, Crowe TD, Nissen SE, Tuzcu EM. Extent and direction of arterial remodeling in stable versus unstable coronary syndromes: an intravascular ultrasound study. *Circulation*. 2000;101:598–603.
10. Brown DL, Hibbs MS, Kearney M, et al. Identification of 92-kD gelatinase in human coronary atherosclerotic lesions: association of active enzyme synthesis with unstable angina. *Circulation*. 1995;91:2125–2131.
11. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*. 1994;94:2493–2503.
12. Halpert L, Sires UL Roby JD, et al. Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. *Proc Natl Acad Sci U S A*. 1996;93:9748–9753.
13. Farb A, Burke AP, Tang AL, et al. Coronary plaque erosion without rupture into a lipid core: a frequent cause of coronary thrombosis in sudden coronary death. *Circulation*. 1996;93:1354–1363.
14. Burke AP, Farb A, Malcom GT, et al. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med*. 1997;336:1276–1282.
15. Burke AP, Farb A, Malcom GT, et al. Effect of risk factors on the mechanism of acute thrombosis and sudden coronary death in women. *Circulation*. 1998;97:2110–2116.
16. Moreno PR, Bernardi VH, Lopez-Cuellar J, et al. Macrophages, smooth muscle cells, and tissue factor in unstable angina: implications for cell-mediated thrombogenicity in acute coronary syndromes. *Circulation*. 1996;94:3090–3097.
17. Toschi V, Gallo R, Lettino M, et al. Tissue factor modulates the thrombogenicity of human

atherosclerotic plaque. *Circulation*. 1997;95: 594–599.
18. Meisel S, Xu XP, Edgington TP, et al. Differentiation of human monocytes into adherent monocyte derived macrophages markedly enhances tissue factor expression and procoagulant activity. [abstract] *Circulation*. 1997;96:11–3708.
19. Giesen PLA, Rauch U, Bohrmann B, et al. Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci U S A*. 1999; 96:2311–2315.
20. Rauch U, Bonderman D, Bohrmann B, et al. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. *Blood*. 2000;96:170–175.
21. Egashira K, Inou T, Hiroka Y, et al. Impaired coronary blood flow response to acetylcholine in patients with coronary risk factors and proximal atherosclerotic lesions. *J Clin Invest*. 1993;91:29–37.
22. O'Brien KD, Allen MD, McDonald TO, et al. Vascular cell adhesion molecule-I is expressed in human coronary atherosclerotic plaques: implications for the mode of progression of advanced coronary atherosclerosis. *J Clin Invest*. 1993;92:945–951.
23. Weis RJ, Pitas RE, Wilson BD, et al. Oxidized low-density lipoprotein increases cultured human endothelial cell tissue factor activity and reduces protein C activation. *FASEB J*. 1991;5:2459–2465.
24. Maser A. Inflammation, atherosclerosis, and ischemic events--exploring the hidden side of the moon [editorial, comment]. *N EngI J Med*. 1997;336:1014–1016.
25. Mendall MA, Patel P, Ballarn L, et al. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ*. 1996;312:1061–1065.
26. Ridker PM, Cushman M, Stampfel MJ, et al. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation*. 1998;97:425–428.
27. Ridker PM, Hennekens CH, Roitman-Johnson B, et al. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet*. 1998;351:88–92.
28. Xu X-P, Meisel SR, Ong JM, et al. Oxidized low density lipoprotein transcriptionally regulates matrix metalloproteinase-9 and its tissue inhibitor in human monocyte-derived macrophages. *Circulation*. 1999;99:993–998.
29. Rajavashisth TB, Liao JK, Galis ZS, et al. Inflammatory cytokines and oxidized low density lipoproteins increase endothelial cell expression of membrane type 1 matrix metalloproteinase. *J Biol Chem*. 1999;274: 11924–11929.
30. Cui MZ, Penn MS, Chisolm GM. Native and oxidized low density lipoprotein induction of tissue factor gene expression in smooth muscle cells is mediated by both Egr-1 and Sp1. *J Biol Chem*. 1999;274:32795–32802.
31. Petit L, Lesnik P, Dachet C, Moreau M, Chapman MJ. Tissue factor pathway inhibitor is expressed by human monocyte-derived macrophages: relationship to tissue factor induction by cholesterol and oxidized LDL. Arterioscler Thromb Vasc Biol. 1999;19:309–315.
32. Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial results: I. Reduction in incidence of coronary heart disease. *JAMA*. 1984;251:351–364.
33. Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of FACAPS/TexCAPS. *JAMA*. 1998;279:1615–1622.
34. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia: West of Scotland Coronary Prevention Study Group. *N Engl J Med*. 1995;16:1301–1307.
35. Manninen V, Elo MO, Frick MH, et al. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. *JAMA*. 1988;260:641-651.
36. Ericsson CG, Hamsten A, Nilsson J, et al. Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet*. 1996;347:849–853.
37. Brown BG, Zhao X-Q, Sacco DE, et al. Lipid lowering and plaque regression: new insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation*. 87:1781–1791, 1993.
38. Aikawa M, Rabkin E, Okada Y, et al. Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content of rabbit atheroma: a potential mechanism of lesion stabilization. *Circulation*. 1998;97:2433–2444.
39. Aikawa M, Rabkin E, Voglic SJ, et al. Lipid lowering promotes accumulation of mature smooth muscle cells expressing smooth muscle myosin heavy chain isoforms in rabbit atheroma. *Circ Res*. 1998;83:1015–1026.
40. Aikawa M, Voglic SJ, Sigiyama S, et al. Dietary lipid lowering reduces tissue factor expression in rabbit atheroma. *Circulation*. 1999;100:1215–1222.
41. Crisby M, Nordin-Fredriksson G, Shah PK, et al. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, matrix metalloproteinases and cell death in human carotid plaques: implications for plaque stabilization. *Circulation*. 2001;103:926-933.
42. Shah PK. Focus on HDL: a new treatment paradigm for athero-thrombotic vascular disease. *Exp Opin Invest Drugs*. 2000;9:2139–2146.
43. Plump AS, Scott CJ, Breslow JL. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E–deficient mouse. *Proc Natl Acad Sci USA*. 1994;91:9607–9611.
44. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature*. 1991;353:265–267.
45. Tangirala RK, Tsukamoto K, Chun SH, et al. Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. *Circulation*. 1999;100:1816–1822.
46. Benoit P, Emmanuel F, Caillaud JM, et al. Somatic gene transfer of human ApoA-I inhibits atherosclerosis progression in mouse models. *Circulation*. 1999;99:105–110.
47. Shah PK, Nilsson J, Kaul S, et al. Effects of recombinant apolipoprotein A-I (Milano) on aortic atherosclerosis in apolipoprotein E–deficient mice. *Circulation*. 1998;97:780–785.
48. Ameli S, Hultgardh-Nilsson A, Cercek B, et al. Recombinant apolipoprotein A-I milano reduces intimal thickening after balloon injury in hypercholesterolemic rabbits. *Circulation*. 1994;90:1935–1941.
49. Dimayuga P, Zhu J, Oguchi S, et al. Reconstituted HDL containing human apolipoprotein A-I reduces VCAM-1 expression and neointima formation following periadventitial cuff-induced carotid injury in apoE null mice. *Biochem Biophys Res Commun*. 1999;264:465-468.
50. 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;96:4095–4103.
51. Mayr M, Kiechl S, Willeit J, Wick G, Xu Q: Infections, immunity, and atherosclerosis: associations of antibodies to *Chlamydia pneumoniae, Helicobacter pylori*, and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis. *Circulation*. 2000;102:833–839.
52. Moazed TC, Kuo C, Grayston JT, Campbell LA. Murine models of *Chlamydia pneumoniae* infection and atherosclerosis. *J Infect Dis*. 1997;175:883–890.
53. Muhlestein JB, Anderson JL, Hammond EH, et al. Infection with *Chlamydia pneumoniae* accelerates the development of atherosclerosis and treatment with azithromycin prevents it in a rabbit model. *Circulation*. 1998;97:633–636.
54. Alber DG, Powell KL, Vallance P, Goodwin DA, Grahame-Clarke C. Herpesvirus infection accelerates atherosclerosis in the apolipoprotein E–deficient mouse. *Circulation*. 200;102:779–785.
55. Hu H, Pierce GN, Zhong G. The atherogenic effects of chlamydia are dependent on serum cholesterol and specific to *Chlamydia pneumoniae*. *J Clin Invest*. 1999;103:747–753.
56. Mallat Z, Besnard S, Duriez M, et al. Protective role of interleukin-10 in atherosclerosis. *Circ Res*. 1999;85:e17–e24.
57. Gupta S, Leatham EW, Carrington D, et al. Elevated *Chlamydia pneumoniae* antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation*. 1997;96;404–407.
58. Gurfinkel E, Bozovich G, Daroca A, et al. Randomized trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS Pilot Study: Roxis Study Group. *Lancet*. 1997;350:404–407.
59. Anderson JL, Muhlestein JB, Carlquist J, et al. Randomized secondary prevention trial of azithromycin in patients with coronary artery disease and serological evidence for *Chlamydia pneumoniae* infection: the Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) Study. *Circulation*. 1999;99:1540–1547.
60. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA*. 1995;274: 1049–1057.
61. Graham IM, Daly LE, Refsum HM, et al. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. *JAMA*. 1997;277:1775–1781.
62. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med*. 1997;337:230–236.
63. Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. *JACC*. 1996;27:517–527.

64. Moghadasian MH, McManus BM, Frohlich JJ. Homocysteine and coronary artery disease, clinical evidence and genetic and metabolic background. *Arch Intern Med.* 1997;157:2299–2308.
65. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med*. 1998;338:1042–1050.
66. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10:111–113.
67. Kluijtmans LAJ, van den Heuvel LP, Boers GH, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 1996;58:35–41.
68. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation*. 1996;93:7–9.
69. Deloughery TG, Evans A, Sadeghi A, et al. Common mutation in methylenetetrahydrofolate reductase, correlation with homocysteine metabolism and late-onset vascular disease. *Circulation*. 1996;94:3074–3078.
70. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation*. 1996;94:2410–2416.
71. Schwartz SM, Siscovick DS, Malinow MR, et al. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. *Circulation*. 1997;96:412–417.
72. Pruefer D, Scalia R, Lefer AM. Homocysteine provokes leukocyte-endothelium interaction by downregulation of nitric oxide. *Gen Pharmacol*. 1999;33:487–498.
73. Bescond A, Augier T, Chareyre C, Garcon D, Hornebeck W, Charpiot P. Influence of homocysteine on matrix metalloproteinase-2: activation and activity. *Biochem Biophys Res* Commun. 1999;263:498–503.
74. Fryer RH, Wilson BD, Gubler DB, Fitzgerald LA, Rodgers GM. Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. *Arterioscler Thromb*. 1993;13:1327–1333.
75. Durand P, Lussier-Cacan S, Blache D. Acute methionine load-induced hyperhomocysteinemia enhances platelet aggregation, thromboxane biosynthesis and macrophage-derived tissue factor activity in rats. *FASEB J*. 1997;11:1157–1168.
76. Khajuria A, Houston DS. Induction of monocyte tissue factor expression by homocysteine: a possible mechanism for thrombosis. *Blood*. 2000;96:966–972.
77. Dardik R, Varon D, Tamarin I, et al. Homocysteine and oxidized low density lipoprotein enhanced platelet adhesion to endothelial cells under flow conditions: distinct mechanisms of thrombogenic modulation. *Thromb Haemost*. 2000;83:338–344.
78. Constans J, Blann AD, Resplandy F, et al. Three months supplementation of hyperhomocysteinaemic patients with folic acid and vitamin B6 improves biological markers of endothelial dysfunction. *Br J Haematol*. 1999;107:776–778.
79. Hackam DG, Peterson JC, Spence JD. What level of plasma homocysteine should be treated? Effects of vitamin therapy on progression of carotid atherosclerosis in patients with homocysteine levels above and below 14 micromol/L. *Am J Hypertens*. 2000;13: 105–110.
80. Vermeulen EGJ, Stehouwer CDA, Twisk JWR, et al. Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomised, placebo-controlled trial. *Lancet*. 2000;355:517–522.
81. Malik FS, Lavie CJ, Mehra MR, Milani RV, Re RN. Renin-angiotensin system: genes to bedside. *Am Heart J*. 1997;134:514–526.
82. Kranzhofer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kubler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 1999;19:1623–1629.
83. Warnholtz A, Nickenig G, Schulz E, et al.: Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. *Circulation*. 1999;99:2027–2033.
84. Puey ME, Gonzalez W, Nicoletti A, et al. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappa B activation induced by intracellular oxidative stress. *Arterioscler Thromb Vasc Biol*. 2000;20:645–651.
85. Keidar S, Kaplan M, Hoffman A, Aviram M. Angiotensin II stimulates macrophage-mediated oxidation of low density lipoprotein. *Atherosclerosis*. 1995;115:201–215.
86. Daugherty A, Manning MW, Cassis LA: Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E–deficient mice. *J Clin Invest*. 2000;105:1605–1612.
87. Campbell JH, Fennessy P, Campbell GR. Effect of perindopril on the development of atherosclerosis in the cholesterol-fed rabbit. *Clin Exp Pharmacol Physiol Suppl*. 1992;19:13–17.
88. Hoshida S, Nishida M, Yamashita N, et al. Vascular angiotensin-converting enzyme activity in cholesterol-fed rabbits: effects of enalapril. *Atherosclerosis*. 1997;130:53–59.
89. Hayek T, Attias J, Smith J, Breslow JL, Keidar S. Antiatherosclerotic and antioxidative effects of captopril in apolipoprotein E–deficient mice. *J Cardiovasc Pharmacol*. 1998;31:540–544.
90. The Heart Outcome Prevention Evaluation Study Investigators. Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high risk patients. *N Engl J Med*. 2000;342:145–153.
91. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J Clin Inves*t. 1999;103:571–577.
92. Palinski W, Rosenfeld ME, Yla-Herttuala S, et al. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A*. 1989;86:1372–1376.
93. Salonen JT, Yla-Herttuala S, Yamamoto R, et al. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet*. 1992;339:883–887.
94. Palinski W, Ord V, Plump AS, Breslow JL, Steinberg D, Witztum J. Apoprotein E–deficient mice are a model for lipoprotein oxidation in atherogenesis: demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler Thromb*. 1994;14:605–616.
95. Bergmark C, Wu R, de Faire U, Lefvert AK, Swedenborg J. Patients with early-onset peripheral vascular disease have increased levels of autoantibodies against oxidized LDL. *Arterioscler Thromb Vasc Biol*. 1995;15:441–445.
96. Wu R, Nityanand S, Berglund L, Lithell H, Holm G, Lefvert AK. Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction. *Arterioscler Thromb Vasc Biol*. 1997;17:3159–3163.
97. Iribarren C, Folsom AR, Jacobs DR Jr, Gross MD, Belcher JD, Eckfeldt JH. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis: a case-control study. The ARIC study investigators. Atherosclerosis Risk in Communities. *Arterioscler Thromb Vasc Biol*. 1997;17:1171–1177.
98. Chiesa R, Melissano G, Castellano R, et al. In search of biological markers of high-risk carotid artery atherosclerotic plaque: enhanced LDL oxidation. *Ann Vasc Surg*. 1998;12:1–9.
99. Lehtimaki T, Lehtinen S, Solakivi T, et al. Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1999;19:23–27.
100. Erkkila AT, Narvanen O, Lehto S, Uusitupa MI, Yla-Herttuala S. Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2000;20:204–209.
101. Uusitupa MI, Niskanen L, Luoma J, Vilja P, Mercuri M, Rauramaa R, Yla-Herttuala S. Autoantibodies against oxidized LDL do not predict atherosclerotic vascular disease in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1996;16:1236–1242.
102. Boullier A, Hamon M, Walters-Laporte E, et al. Detection of autoantibodies against oxidized low-density lipoproteins and of IgG-bound low density lipoproteins in patients with coronary artery disease. *Clin Chim Acta*. 1995;30:238:1–10.
103. Fukumoto M, Shoji T, Emoto M, Kawagishi T, Okuno Y, Nishizawa Y. Antibodies against oxidized LDL and carotid artery intima-media thickness in a healthy population. Arterioscler Thromb Vasc Biol. 2000;20:703–707.
104. Nicoletti A, Kaveri S, Caligiuri G, Bariety J, Hansson GK. Immunoglobulin treatment reduces atherosclerosis in apo E knockout mice. *J Clin Invest*. 1998;102:910-918
105. Ameli S, Hultgardh J, et al. Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol*. 1996;16:1074–1079.
106. George J, Afek A, Gilburd B, et al. Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis*. 1998;138:147–152.
107. Freigang S, Horkko S, Miller E, Witztum JL, Palinski W. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes. *Arterioscler*

Thromb Vasc Biol. 1998;18:1972–1982.
108. Gupta S, Pablo AM, Jiang XC, Wang N, Tall AR, Schindler C. IFN-gamma potentiates atherosclerosis in apo E knock-out mice. *J Clin Invest*. 1997;99:2752–2761.
109. Lee TS, Yen HC, Pan CC, Chau LY. The role of interleukin 12 in the development of atherosclerosis in apoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 1999;19:734–742.
110. Lentsch AB, Shanley TP, Sarma V, Ward PA. In vivo suppression of NF-Kappaβ and preservation of I Kappaβ alpha by interleukin-10 and interleukin-13. *J Clin Invest*. 1997;100:2443–2448.
111. Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J Clin Invest*. 1995;96:2304–2310.
112. Jungi TW, Brcic M, Eperon S, Albrecht S. Transforming growth factor-β and interleukin-10, but not interleukin-4, down-regulate procoagulant activity and tissue factor expression in human monocyte-derived macrophages. *Thromb Res*. 1994;76:463–474.
113. Oslund LJP, Hedrick CC, Olvera T, et al. Interleukin-10 blocks atherosclerotic events in vitro and in vivo. *Arterioscler Thromb Vasc Biol*. 1999;19:2847–2853.