Prevention of Plaque Rupture: A New Paradigm of Therapy

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Acute coronary syndromes—unstable angina, myocardial infarction, and sudden cardiac death—are caused by acute disruption of an unstable coronary atheroma. Unstable plaques have three histologic characteristics: a large lipid core, many inflammatory cells, and a thin fibrous cap. Because the unstable plaque is not necessarily obstructive, it may cause no symptoms before rupture. The cellular processes that lead to the characteristic histologic features of unstable plaque have recently been identified. This new understanding of the cell biology of plaque instability suggests new therapeutic strategies: passivation of the endothelium, reduction of low-density lipoprotein (LDL) in the vessel wall by decreasing serum LDL levels or accelerating reverse cholesterol transport, inhibition of LDL oxidation, inhibition of inflammatory cytokine expression, and inhibition of thrombus formation. Although the morbidity and mortality resulting from acute coronary disease have been reduced by more than 50% over the past 30 years, it is reasonable to anticipate further reductions of similar magnitude in the decade ahead.

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Clinical strategies for preventing acute coronary syndromes now focus on preventing plaque instability. The concept has evolved over several decades. Beginning in the 1980s, angioscopy revealed atheroma with a smooth surface in patients with stable angina but showed atheroma with a disrupted intimal surface in patients with acute coronary syndromes (**Figure 1**) (1). Pathologists subsequently demonstrated that disrupted lesions have three characteristic histologic features: a large lipid core, an abundance of inflammatory cells, and a thin fibrous cap (2, 3). Angiographers found that these unstable lesions are not necessarily severely stenotic (4). Finally, in the middle and late 1990s, plaque instability was even more clearly defined when cytokines and enzymes that cause plaque instability at the level of cytokines were identified (**Figure 2**). In this article, I describe the steps in plaque destabilization and link them to a set of clinical strategies that can be expected to substantially decrease the incidence of acute coronary syndromes.

Mechanisms Responsible for Evolution of Unstable Plaque

Endothelial Activation

The process of plaque destabilization begins with endothelial activation (**Figure 3**). Some of the endothelial cell activators are the familiar traditional risk factors (increased cholesterol levels, hypertension, and smoking), whereas others (for example, homocysteinemia, immune complexes, and other infectious agents) have been identified more recently (5, 6). The level of endothelial cell activation can change very rapidly (7, 8). For instance, Mellwig and colleagues (8) found a 30% increase in stress-induced coronary blood flow 20 hours after low-density lipoprotein (LDL) apheresis, which lowered mean LDL levels from 194 to 81 mg/dL (5.0 to 2.0 mmol/L), and Vogel (6) found substantial reduction in forearm vascular reactivity in a 4-hour period after a fatty meal. The activated endothelial cell expresses cell adhesion molecules, which cause circulating blood cells to adhere to the endothelium and enter the blood vessel wall. Animals deficient in adhesion molecules have 40% smaller lesions than those with normal production (9). Adhesion molecules are found on the surface of human atheroma (10) and are strongly associated with increased intimal leukocyte accumulation. Serum levels of adhesion molecules correlate directly with carotid intimal thickness (11).

Formation of the Lipid Core

As cells enter the vessel wall, cholesterol also enters as LDL. Macrophages avidly take up cholesterol from apolipoprotein B–containing lipoproteins and are trapped in the subendothelial space, forming the lipid core. The anatomic differences between stable and disrupted plaques are striking. In aortae with disrupted plaque, unstable lesions have a much greater cross-sectional area occupied by lipid (64% vs. 14%), an eightfold greater area occupied by macrophages, and a thinner fibrous cap (0.13 mm vs. 0.44 mm) (12).

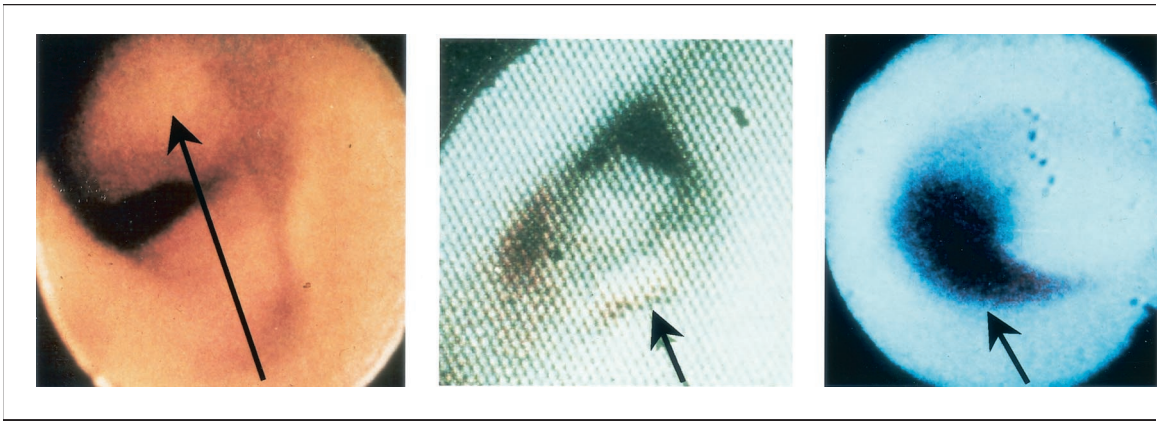
Reverse Cholesterol Transport

Cholesterol in the blood vessel wall is transferred to high-density lipoprotein (HDL) (13–15) for transport back to the liver for excretion in the bile. Reverse cholesterol transport involves cholesterol transfer from cell membranes to phospholipid acceptor particles, the most important of which are nascent HDL particles and two proteins. Cholesterol in the nascent HDL is esterified by lecithin cholesterol acyltransferase to cholesterol esters, and cholesteryl ester transfer protein exchanges the ester for triglyceride. Because HDL also inhibits adhesion molecule expression (16), is an antioxidant (17), and blocks matrix metalloproteinase expression, the antiatherogenic mechanisms through which HDL act are not firmly established.

LDL Oxidation

The native LDL that enters the blood vessel wall undergoes oxidation by oxygen free radicals (18). The uptake of oxidized LDL by macrophages stimulates expression of cytokines and proteolytic enzymes. In humans, antibodies against LDL are found in atherosclerotic lesions, and plasma contains antibodies that react with oxidized LDL

Figure 1. **The angioscopic appearance of stable and unstable atheroma.**



Left. The typical smooth surface of a stable atheroma in a patient with stable angina. **Middle.** The disrupted intimal surface of an atheroma in a patient with unstable angina. **Right**. A thrombus on the surface of a disrupted atheroma in a patient with acute myocardial infarction.

(19). Furthermore, the level of plasma-oxidized LDL increases with age and is substantially higher in patients with atherosclerosis than in controls (20).

Cell Activation in the Vessel Wall

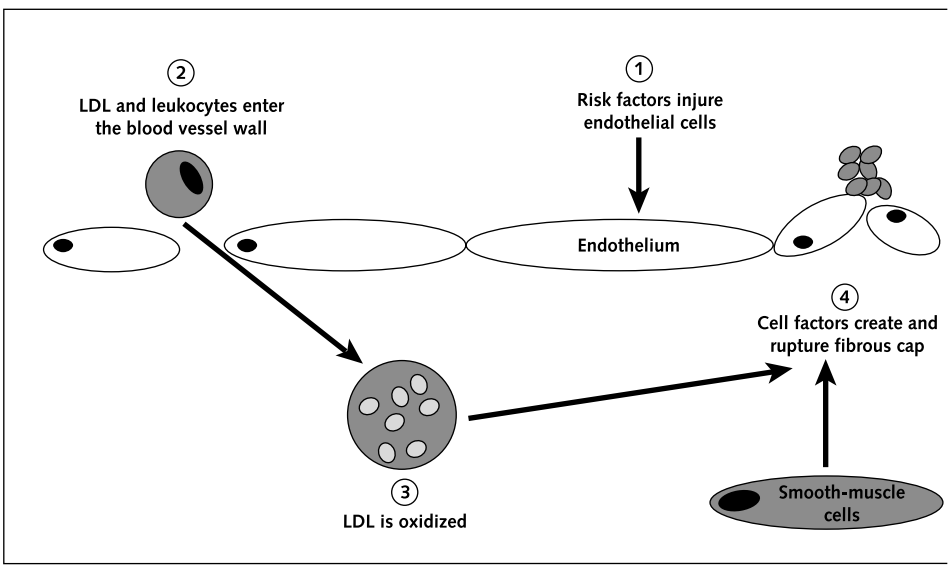
In an unstable plaque, every cell type is activated. This process is strikingly similar to that of other chronic inflammatory conditions, such as rheumatoid arthritis (21). The smooth-muscle cell changes its phenotype, developing twice the volume of secretory granules as the normal quiescent cell (22). The blood monocyte becomes a tissue macrophage (23). The mast cell becomes positive for tumor necrosis factor-α, and the percentage of mast cells that are degranulated increases 15- to 20-fold (24, 25). The T lymphocyte is also activated (26).

A spectrum of cytokines with many overlapping actions determine whether the individual atheroma progresses to stability or to instability (27–30). For instance, tumor necrosis factor-α activates endothelial cells, transforming growth factor-β stimulates the production of lipoprotein-trapping proteoglycans, colony-stimulating factors cause macrophage replication, and interferon-γ suppresses smooth-muscle replication (26, 31).

Thinning and Rupture of the Fibrous Cap

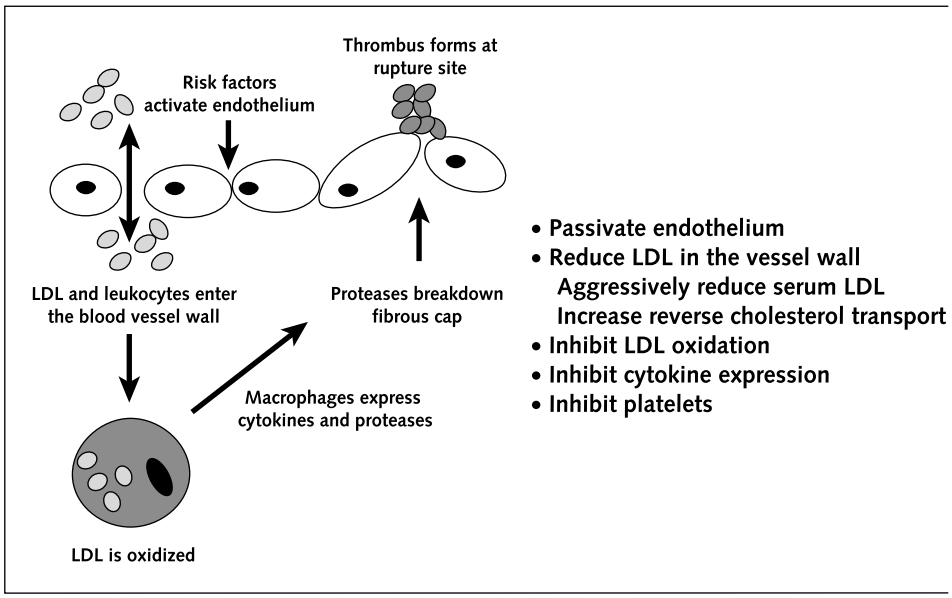
Smooth-muscle cells produce collagen and other extracellular matrix proteins, which provide mechanical strength, whereas activated inflammatory cells, particularly macrophages and mast cells, express enzymes that degrade the collagen (32, 33). In unstable plaque, the balance

Figure 2. **The steps in atheroma destabilization.**



Activated endothelial cells express adhesion molecules, which attract leukocytes that enter the blood vessel wall. Low-density lipoprotein (*LDL*) in the vessel wall is oxidized and taken up by macrophages. The activated cells in the vessel wall express cytokines, which maintain the inflammatory process. Proteases digest the fibrous cap, and smooth-muscle cells undergo apoptosis, leading to rupture of the fibrous cap.

Figure 3. **The biological processes that induce plaque destabilization and the therapeutic strategies that inhibit these processes.**



If the processes are relatively independent, the therapies may have an additive effect. LDL = low-density lipoprotein.

among these competing factors favors collagen breakdown rather than synthesis.

Thinning of the fibrous cap involves at least two processes: increased collagen breakdown by proteases and decreased collagen synthesis by smooth-muscle cells. The family of metalloproteinases, expressed by macrophages, erodes the fibrous cap (34). The expression of metalloproteinases is driven by cytokines, such as tumor necrosis factor-α and interleukin-1, which upregulate macrophage metalloproteinase activity (35–37). Oxidized LDL doubles metalloproteinase expression, whereas native LDL has no effect (38, 39). In addition, collagen synthesis is diminished as a result of both decreased function and apoptosis of the smooth-muscle cell (40, 41). The decrease in the smooth-muscle cell population in unstable plaques reflects increased expression of cytokines, such as tumor necrosis factor-α and interferon-γ, which induce apoptosis.

Remarkably, the extracellular matrix itself helps destroy the fibrous cap. Wallner and colleagues found that the extracellular protein tenascin-C, which induces metalloproteinase expression and causes smooth-muscle cell apoptosis, is not present in the normal vessel wall but is strongly expressed in unstable plaque (42, 43).

Unstable plaques most commonly rupture at the plaque shoulder, where T lymphocytes and macrophages predominate and smooth-muscle cells are less common. Tissue factor content is increased twofold in unstable plaques, which promotes thrombosis when the plaque ruptures (44). **Table 1** summarizes the best-studied cytokines and their effects.

The Contribution of Systemic Inflammation to Local Plaque Instability

An accumulating body of recent evidence suggests that systemic factors may influence local plaque instability. Compared with stable angina, the temperature of culprit lesions is 0.6 °C higher in unstable angina and 1.0 °C higher in myocardial infarction. The plaque's temperature correlates with its macrophage density. Local plaque temperature also correlates with the systemic level of circulating cell adhesion molecules, cytokines, and plasma C-reactive protein (45, 46). These data suggest an interaction

Table 1. **Destabilizing Effects of Cell Products Identified in Unstable Atheroma***

Cell Product (Reference)	Important Action in Unstable Plaque	Other Actions
Tumor necrosis factor-α (36)	Upregulates adhesion molecules	Increases thrombogenicity
Interleukin-1β (36)	Activates endothelial cells	Causes smooth-muscle cell apoptosis
Matrix metalloproteinase (29)	Digests collagen	Digests elastin
Tenascin (42)	Stimulates matrix metalloproteinase expression	Causes smooth-muscle cell apoptosis
Transforming growth factor-β (31)	Stimulates collagen synthesis	Stimulates lipid-trapping proteoglycans
Tissue factor (44)	Promotes thrombin generation	Promotes matrix metalloproteinase expression
Insulin-like growth factor-δ (41)	Suppresses collagen expression	Causes smooth-muscle cell apoptosis

* Each cell product in unstable plaque has multiple actions that contribute to stabilization. In addition, there is substantial overlap among the effects of cytokines. Because of this redundancy, it is unlikely that targeting a single cytokine will be an effective approach.

Table 2. **Key Points***

Unstable plaques cause acute coronary syndromes.
Local inflammation causes plaque instability.
Systemic inflammation augments local inflammation.
Plaque destabilization proceeds through five steps: endothelial activation, LDL entry into blood vessel walls, LDL oxidation, breakdown of the fibrous cap, and thrombus formation.
Each step of plaque destabilization can be inhibited by preventive therapies.
The incidence of acute coronary syndromes can be substantially reduced.

*LDL = low-density lipoprotein.

between systemic and local inflammatory processes. Danesh (47) performed a meta-analysis of seven studies involving 1053 cases of nonfatal myocardial infarction or death related to coronary heart disease; the mean follow-up in these studies was 6 years. The risk ratio of coronary heart disease for persons in the upper tertile of plasma C-reactive protein compared with those in the bottom tertile was 1.7 (47). Similar relationships have been found for other markers of systemic inflammation, such as fibrinogen and amyloid A. It is as yet unclear whether these indicators of systemic inflammation are a marker or a mediator of local plaque instability.

The Biological Basis of Unstable Plaque Histology

Returning to the three histologic characteristics of unstable plaque, the pathogenesis of each feature can now be defined. The large lipid core results from the entry of lipids and monocytes into the blood vessel wall, a process initiated and maintained by endothelial cell activation. The monocytes are transformed into tissue macrophages by ingestion of oxidized LDL, creating the lipid core. The abundance of inflammatory cells in the unstable plaque results from their attraction to the vessel wall by adhesion molecules and is maintained and promoted by subsequent cytokine-induced cell activation. The thin fibrous cap results from extracellular matrix breakdown that is driven by protease expression induced by both the activated cells and the extracellular matrix. Diminished collagen synthesis as a result of smooth-muscle cell apoptosis occurs concurrently.

From this detailed description of the cell biology of plaque destabilization, it is now possible to extract several broad generalizations (**Table 2**). The fundamental cause of plaque instability is the imbalance of activation and passivation of the cells of the blood vessel wall, that is, inflammation. The final common pathway is the predominance of collagen breakdown over collagen synthesis in the fibrous cap. And, quite unexpectedly, the extracellular matrix is both a target and a modulator in plaque destabilization.

Therapeutic Strategies Derived from Cell Biology

The cell biology of plaque destabilization suggests that many routes, other than lowering LDL levels, may provide stabilization. Both epidemiologic data and recent clinical trials support this possibility. Data collected prospectively over 25 years on the relationship between serum cholesterol level and cardiac death in different countries show that within each quartile of serum cholesterol level, cardiac mortality differs as much as fourfold (48). Although this difference in major adverse cardiac events at the same cholesterol level may reflect variations in diet, lifestyle, environment, or genetics, its magnitude indicates the importance of factors beyond blood cholesterol. Reductions in cardiac events without lowering cholesterol levels support this idea.

Within the limited scope of this article and with the understanding that therapies often affect several mechanisms, the biology of plaque instability suggests at least five new strategies (**Figure 3**): passivation of the endothelium, reduction of LDL in the vessel wall by aggressively reducing serum LDL levels or accelerating reverse cholesterol transport, inhibition of LDL oxidation, inhibition of inflammatory cytokine and protease expression, and inhibition of platelets.

Suppression of Endothelial Activation: The Role of Angiotensin Inhibition and Fish Oil

Endothelial activation can be viewed somewhat simplistically as the result of the balance between two of the most important factors that affect endothelial function—angiotensin II and nitric oxide. When the balance is shifted toward angiotensin II, endothelial activation results, with the potential to initiate plaque destabilization. Angiotensin alters the blood vessel wall through many biological mechanisms, only one of which is endothelial activation and promotion of adhesion molecule expression (49). For instance, angiotensin also promotes formation of oxygen free radicals (50) and regulates extracellular matrix function.

Angiotensin-converting enzyme (ACE) inhibitors reduce atherosclerosis in many nonhypertensive animal models, including primates (51). Although the mechanisms for the antiatherosclerotic effect are undefined and probably multiple, ACE inhibitors do reduce adhesion molecule expression and macrophage infiltration in the rabbit model of early accelerated atherosclerosis (52). In Cynomolgus monkeys, Song and colleagues (53) reported that the area of aortic atherosclerosis decreased from 58% to 24% with ACE inhibition; this finding was accompanied by a marked decrease in angiotensin I and II receptor density to normal levels. A similar magnitude of reduction in atherosclerosis has been confirmed with other ACE inhibitors (54). The first randomized, placebo-controlled trial to demonstrate reduction of major adverse cardiac events in normotensive humans is the Heart Outcomes Prevention Evaluation study (55). Ramipril (10 mg orally) or placebo was given for 5 years to 9297 patients without heart failure who were at high risk for a cardiac event (>54 years of age, vascular disease or diabetes, and one additional risk factor). In the treatment group, the event rate was reduced by 22%, sug-

gesting that angiotensin II inhibition has potent antiatherogenic effects. Several ongoing trials seek to confirm the hypothesis. In one of these trials, 14 000 patients with coronary heart disease who have normal left ventricular function are being treated with trandolapril (4 mg/d) added to standard therapy over 5 years; the primary end point is cardiovascular mortality and myocardial infarction.

Angiotensin receptor blockers reduce the development of atherosclerosis in hypercholesterolemic primates. The results of clinical trials have not yet been reported. However, in a study by Navalker and colleagues, patients with normotensive stable coronary disease who were treated with irbesartan (70 to 150 mg/d for 24 weeks) had a decrease in soluble adhesion molecule (36%), tumor necrosis factor-α (54%), and superoxide levels (52%). Because irbesartan did not alter blood pressure in these normotensive patients, the results suggest that the drug independently retarded the inflammatory component of atherosclerosis (56).

Marine n-3 polyunsaturated fatty acids decrease very-low-density lipoprotein (VLDL) and triglyceride levels and elevate HDL levels. In addition, fish oils have modest antihypertensive, antithrombotic, and antiarrhythmic activity (57). Most epidemiologic studies have not shown a relationship between fish intake and a reduction in the prevalence of coronary heart disease. Nonetheless, randomized trials have demonstrated positive effects from administering fish oil after infarction. Both the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Prevenzione trial (58) and the Diet and Reinfarction Trial (59) observed 10% to 15% reductions in major adverse cardiac events over a 5-year follow-up period. The advice to eat one or more fish meals weekly is reasonable, particularly if these meals are substitutes for saturated fats in animal meats.

Reduction of LDL Entry: The Role of Statins

Substantial lowering of blood lipid levels leads to plaque stabilization. Aikawa and colleagues found that a 16-month lipid-lowering diet fed to hypercholesterolemic rabbits progressively reduced vascular macrophage content by 20-fold. Metalloproteinase immunoreactivity became almost undetectable (60), and interstitial collagen content increased substantially.

New statin dose formulations that permit routine LDL lowering to less than 80 mg/dL (2 mmol/L) raise the issue of very aggressive LDL lowering (61), particularly because atherosclerosis is rare to absent in animals and humans with total serum cholesterol levels less than 160 mg/dL (<4 mmol/L). On the other hand, in epidemiologic studies, the slope of the relationship between major adverse cardiac events and LDL may have an inflection point close to an LDL level of 100 mg/dL (2.5 mmol/L), which strongly suggests a progressively smaller therapeutic benefit as the LDL level is lowered to less than 100 mg/dL. The data from clinical trials are contradictory, possibly reflecting the multiplicity of factors that influence risk and outcome or different burdens of atherosclerotic disease.

In the Cholesterol and Recurrent Events study, patients with a pretreatment LDL level less than 125 mg/dL (<3.2 mmol/L) had no reduction in major adverse cardiac events with statin therapy (62). A subgroup analysis of the West of Scotland Coronary Prevention study (63) also showed no further reduction in coronary heart disease risk beyond a 24% reduction in LDL levels. In contrast, the Post Coronary Artery Bypass Graft trial, which compared LDL lowering to targets of either 95 mg/dL (2.4 mmol/L) or 135 mg/dL (3.5 mmol/L) (64), reported substantially better angiographic and clinical outcomes in the aggressively treated cohort. This benefit increased as duration of follow-up increased from 5 to 8 years. Subgroup analysis in this trial and several other trials have suggested progressively greater benefit at lower levels of on-treatment LDL.

Several ongoing trials are addressing the issue of aggressive LDL lowering. The Treat to New Targets trial has randomly assigned 10 004 patients with coronary heart disease and LDL levels of 130 to 250 mg/dL (3.3 to 6.4 mmol/L) to either 10 mg or 80 mg of atorvastatin; the end point is death or myocardial infarction at 5-year follow-up. This strategy will allow comparison of on-treatment LDL levels of approximately 70 to 80 mg/dL (1.8 to 2.1 mmol/L) to 90 to 110 mg/dL (2.3 to 2.8 mmol/L). The Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE IT) trial, which is sponsored by the manufacturers of pravastatin, aims to demonstrate that there is no difference in clinical outcomes as LDL levels are lowered beyond those close to the current National Cholesterol Education Program guideline. In contrast, the Reversal of Atherosclerosis with Lipitor trial, sponsored by the manufacturers of atorvastatin, aims to show plaque regression in 600 patients who are using the same drug dosages (80 mg of atorvastatin vs. 40 mg of pravastatin) as those being used in the PROVE IT trial; the end point is a coronary intravascular or angiographic event at 18 months. These studies should contribute substantially to defining the value, if any, of aggressive LDL lowering.

Statins, like ACE inhibitors, have pleiotropic anti-inflammatory effects independent of their perceived primary action (**Table 3**). For instance, statins decrease monocyte adhesion independent of cholesterol lowering (65). Animal studies show that LDL lowering with statins (similar to lowering with diet) reduces plaque macrophage and cholesterol ester content, increases the volume of collagen and smooth-muscle cells, and reduces platelet activation and blood hyperviscosity. In a nonrandomized study of 24 patients undergoing carotid endarterectomy, patients were given either statin therapy for 3 months or no lipid-lowering therapy. Atheroma in patients given statin therapy had 75% less lipid core, 40% less oxidized LDL and metalloproteinase, and twice the amount of collagen (66). Statin therapy also reduces thrombin generation by 40% in patients with hypercholesterolemia (67). These pleiotropic

Table 3. **Pleiotropic Actions of Antiatherosclerotic Therapies***

Therapy	Action					
	Improve Endothelial Function	Decrease Low-Density Lipoprotein Levels	Inhibit Low-Density Lipoprotein Oxidation	Increase Reverse Cholesterol Transport	Reduce Inflammation	Inhibit Thrombosis
Angiotensin-converting enzyme inhibitor and angiotensin receptor blocker	X	–	X	–	X‡	–
Statin	X	X	X	†	X‡	–
Vitamin E	X	–	X	–	X‡	–
Fish oil	–	X	–	†	–	X
Increase in high-density lipoprotein levels	X	–	X	X	X	–
Fibrates	X	–	–	†	–	–
Aspirin	–	–	–	–	X	X
Clopidogrel	–	–	–	–	–	X

* Almost all of the methods for reducing plaque instability have many actions on endothelial function, low-density lipoprotein levels, oxidation, reverse cholesterol transport, inflammation, and thrombosis. Dash = published data are not available, but there is not a good basis for suspecting an effect; X = promotes effect.
† Statins, fish oil, and fibrates can increase high-density lipoprotein levels and might thereby accelerate reverse cholesterol transport.
‡ The anti-inflammatory action may result in part from the antioxidant effect.

effects may explain some of the beneficial effects of long-term statin therapy.

Initiation of aggressive therapy to lower LDL levels during hospitalization in patients with acute coronary syndromes may contribute to early plaque stabilization. In the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering study (68), treatment with 80 mg of atorvastatin daily was begun during hospitalization and patients were followed through 16 weeks. The average LDL level declined by 40% to 72 mg/dL (1.9 mmol/L) with atorvastatin, but the placebo group was unchanged (68). Compared with the placebo group, the atorvastatin group had a 16% reduction in the composite primary end point event (14.8% vs. 17.4% [$P = 0.048$]), primarily resulting from reduced re-hospitalization for recurrent symptomatic myocardial ischemia. The atorvastatin group had a slight increase in reversible elevation of liver function test results; there were no cases of myositis. On the basis of these and other confirming studies, most cardiologists favor in-hospital initiation of statin therapy in patients with acute coronary syndromes.

LDL levels can also be reduced by preventing dietary cholesterol absorption. Cholesterol acyltransferase facilitates cholesterol absorption from the intestine and is in part responsible for the formation and accumulation of cholesterol esters in the vessel wall. In hamsters, acyl-coenzyme A cholesterol acyltransferase inhibitors reduced cholesterol fatty streak area by 90% after 8 weeks (69). The effect seems to be synergistic with statins. In cholesterol-fed rabbits, Bocan and colleagues found that doses of atorvastatin and an acyl-coenzyme A cholesterol acyltransferase inhibitor that reduced plasma cholesterol levels by 29% and 39%, respectively, resulted in a 60% reduction when administered concurrently (70). The reduction of plasma cholesterol was paralleled by similar reductions in atheroma mass and macrophage area.

Ezetimibe is a specific inhibitor of the receptor responsible for transfer of cholesterol from intestinal lumen into the intestinal wall. Preliminary studies suggest that ezetimibe when used alone lowers LDL levels by 15% to 20% but when used in combination with statins can reduce LDL levels by 60%.

Acceleration of Reverse LDL Cholesterol Transport: The Role of Apolipoprotein A-I, Fibrates, and Niacin

Laboratory data on animals suggest that raising the serum level of HDL or apolipoprotein A-I has several important antiatherosclerotic effects, the most important of which may be the effect on reverse cholesterol transport. Overexpression of human apolipoprotein A-I reduces the progression of atherosclerosis in mice (71, 72). Repeated injections of both purified apolipoprotein A-I (73) and HDL (74) inhibit the development of atherosclerosis in cholesterol-fed rabbits and in apolipoprotein E–deficient mice with an accelerated form of atherosclerosis (75). Antibodies to cholesterol ester transfer protein have been reported to increase HDL levels by 35% and decrease plaque area by 40% in the hypercholesterolemic rabbit (76). Gene transfer of the apolipoprotein A-I gene to the liver of hypercholesterolemic mice induced a 69% increase in apolipoprotein A-I levels, a 54% increase in HDL levels, and a 70% decrease in aortic lesion area (77, 78). The change in lesion area is accompanied by a substantial decrease in lesion macrophage volume.

No currently available clinical therapy increases HDL levels in a magnitude comparable to the statin effect on LDL lowering. Nonetheless, modest increases in HDL associated with triglyceride lowering, with both niacin and fibrates, reduce cardiac event rate. In the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention trial, 1264 of 2531 men with coronary heart disease, an HDL level of 40 mg/dL (1.0 mm/L) or less, and an LDL level of 140 mg/dL (3.6 mmol/L) or less were assigned to receive gemfibrozil (1200 mg/d) for 5 years (79). Triglyceride levels decreased 31%, and HDL levels increased 6%. Although LDL levels did not change, the cardiac event rate

decreased by 22%. Thus, therapies that substantially increase HDL levels hold major promise.

Inhibition of the Oxidative State: The Role of Vitamins and Diet

Vitamins have well-established ex vivo antioxidant properties. The principal antioxidants that protect the LDL molecule from oxidation are the lipid-soluble agents α-tocopherol, γ-tocopherol, and β-carotene (80, 81). Ascorbic acid regenerates reduced α-tocopherol after it has been oxidized. Vitamin E supplements inhibit atherogenesis in animals, including primates (82). In the Cambridge Heart Antioxidant Study, administration of vitamin E, 400 to 800 mg daily, to postinfarction patients was associated with a 41% reduction in the subsequent rate of myocardial infarction, although overall mortality was unchanged (83). Two larger randomized trials, however, have failed to demonstrate a beneficial effect of vitamin E. In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Prevenzione trial, 11 324 patients who recently had a myocardial infarction were randomly assigned to receive vitamin E (300 mg/d), n-3 polyunsaturated acid (1 g/d), both, or neither. Although n-3 polyunsaturated fatty acids reduced major adverse cardiac events by 10% to 14% over 3.5 years, vitamin E had no beneficial effect (84). This result was subsequently supported by the Heart Outcomes Protection Evaluation study, in which 8541 high-risk patients were randomly assigned to receive either vitamin E (400 IU/d) or placebo over a 4.5-year period (85). The number of nonfatal myocardial infarctions, strokes, and deaths from cardiovascular causes did not differ. Although less extensively studied, treatment with vitamin A and vitamin C has produced similar negative results (6). Given the central role that oxygen radicals and oxidized LDL play in the pathogenesis of atherosclerosis in the animal model, the failure of antioxidant vitamin supplementation to reduce major adverse cardiac events in humans is largely unexplained.

Another potentially therapeutic vitamin is folic acid because of its effect on homocysteine levels. As many as 20% of patients with coronary heart disease have elevated levels of homocysteine (86). High homocysteine levels predict cardiovascular and noncardiovascular death. In a population-based cohort of 4766 Norwegian men and women (range, 65 to 67 years of age), homocysteine levels of 9 μmol/L were significantly associated with a 50% increase in cardiovascular death (87). Other investigators have demonstrated that vitamin B and folic acid decrease homocysteine levels; however, to date, clinical trials have not demonstrated that lowering homocysteine levels reduces major adverse cardiac events (88).

Although antioxidant vitamins have thus far failed, we know that diets with antioxidant properties substantially reduce major adverse cardiac events after acute myocardial infarction. The three large randomized trials that have examined the effect of diet in patients with coronary heart disease have shown substantial reductions in major adverse cardiac events (89–91). The Lyon Diet Heart study randomly assigned 605 patients who had experienced their first myocardial infarction to a Mediterranean-type or a prudent western diet; the follow-up period was 4 years (89). There were 95 cardiovascular events in the Mediterranean diet group compared with 180 events ($P < 0.001$) in the prudent western diet group. High blood pressure and cholesterol levels were independent and joint predictors of major adverse cardiac events, thus indicating that the Mediterranean diet did not alter the usual relationships between risk factors and major adverse cardiac events. The many differences between the Mediterranean diet and the prudent western diet preclude any attempt to identify a predominant mechanism. Within this limitation, however, a leading candidate for efficacy in the Mediterranean diet is the n-3 class of essential fatty acids—particularly α-linolenic acid (92).

Inhibition of Inflammatory Cytokines: The Role of Antibiotics and Aspirin

An association between *Chlamydia pneumoniae* and atherosclerosis is suggested by seroepidemiologic studies, which show that patients with coronary heart disease frequently have antibodies against *Chlamydia* species (93) and that atheroma frequently contains these species (94). In 1997, a small English study suggested that azithromycin reduced cardiac events in survivors of myocardial infarction (95, 96). Two subsequent randomized clinical trials of antibiotics have failed to reproduce these results (97, 98). In the Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia study, 302 patients who were randomly assigned to receive placebo or azithromycin for 3 months and then followed for 2 years had no differences in cardiac events (99). Approximately 3500 patients are needed to show a 20% reduction in event rate; two adequately powered trials of coronary artery disease are under way, with results expected in late 2003 (100, 101).

Compared with controls, patients with unstable angina have more than twice the blood levels of interleukin-6, C-reactive protein, and macrophage colony-stimulating factor (102), and these levels decrease after 6 weeks of aspirin treatment. These data, taken together with data from previous studies demonstrating the efficacy of aspirin in reducing early posthospitalization cardiac events, suggest that the anti-inflammatory effect of aspirin may be additive to its antithrombotic effect in patients with plaque instability.

Inhibition of cytokine expression can be directed at a specific cytokine or, more generally, at the inflammatory process. It is possible to inhibit metalloproteinases in vivo. Tissue inhibitors of metalloproteinases naturally antagonize the action of metalloproteinase (103, 104). George and colleagues (104) overexpressed tissue inhibitor of metalloproteinase-3 at the luminal surface of porcine saphenous veins before carotid artery grafting. Neointima

formation was reduced 58%, and metalloproteinase expression was reduced throughout the vessel wall. To date, there are no reported trials of tissue inhibitors of metalloproteinase for preventing plaque instability. Furthermore, the practicality of systemic suppression of metalloproteinases and specific cytokines is problematic because they participate in normal tissue functions.

Inhibition of Thrombosis: The Role of Aspirin and Clopidogrel

Aspirin reduces cardiac events in patients with acute coronary syndromes and in patients not known to have coronary heart disease. The Italian Primary Prevention Project compared low-dose aspirin (100 mg/d) to no therapy in a randomized trial of 4495 persons with one or more cardiovascular risk factors (hypercholesterolemia, diabetes, hypertension, obesity, family history of coronary heart disease, or older age) (105). The trial was terminated at 3.6 years when evidence from two other trials showed benefit in primary prevention. In the Italian study, aspirin reduced the frequency of major adverse cardiac events from 8.2% to 6.3%. Severe bleeding was more frequent in the aspirin group (1.1% vs. 0.3%), and one bleeding complication was fatal in the 8000 person-treatment years. As a result of these findings, cardiologists typically recommend low-dose aspirin for primary prevention of coronary heart disease in persons who have one or more risk factors when blood pressure is not elevated.

In the Clopidogrel in Unstable Angina to Prevent Recurrent Events trial, 12 562 patients received aspirin alone or aspirin plus clopidogrel (75 mg/d) (106). At 9-month follow-up, the clopidogrel group experienced a 20% reduction in cardiac events; the absolute increase in major bleeding was 1%. These results contrast with the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events trial, which was conducted in 19 185 patients with a history of recent stroke, myocardial infarction, or symptomatic peripheral artery disease. Over a 1- to 3-year follow-up period, cardiac events were reduced from 5.8% to 5.3%, a relative risk reduction of only 9% (107).

The multiple potential directions for prevention of plaque instability raise important practical questions. One critical question is the increment of benefit, if any, to be obtained from therapies added to diet and LDL lowering. Clinical trials to date provide little insight. One ongoing trial compares the current standard for optimal medical therapy to angioplasty plus optimal medical therapy. The trial is being conducted in 3260 patients with known coronary heart disease who are being followed for 3 years. Optimal medical therapy includes a statin, an ACE inhibitor, aspirin, a β-blocker, and a calcium-channel blocker, as is appropriate for patients with stable angina. For patients with unstable angina, optimal therapy also includes nitrates, a glycoprotein 2B/3A inhibitor, and heparin. The primary end points are death, myocardial infarction, and refractory angina necessitating bypass surgery. Extensive data on cost-effectiveness and quality of life are also being collected.

CONCLUSION

Cell biology has created a new paradigm for prevention of acute coronary syndromes resulting from plaque rupture. The five new therapeutic directions for plaque stabilization are endothelial passivation, reduction of LDL in the vessel wall by very aggressive LDL lowering or acceleration of reverse cholesterol transport, inhibition of LDL oxidation, inhibition of inflammatory cytokine and protease expression, and inhibition of platelets. Each of these pathways can be affected by currently available therapies. If their effects are additive, major further reductions in coronary events will be possible in the future.

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