[Frontiers in Bioscience 8, s1371-1383, September 1, 2003]

HOW DOES DIABETES ACCELERATE ATHEROSCLEROTIC PLAQUE RUPTURE AND ARTERIAL OCCLUSION?

Stephen M. Schwartz and Karin E. Bornfeldt

Department of Pathology, University of Washington School of Medicine, Seattle WA 98195-7470

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Increased cardiovascular mortality in patients with diabetes
3.1. What is the role of hyperglycemia?
3.2. What is the role of dyslipidemia?
3.3. What is the role of hypertension?
4. How does diabetes affect lesion formation and progression?
4.1. Theories of lesion formation and progression
4.1.1. Lesion initiation
4.1.2. Lesion progression
4.2. Non-diabetic animal models of plaque rupture and occlusion
5. What cellular events are likely to be involved in plaque rupture and erosion and how does diabetes affect these events?
5.1. Inflammation
5.2. Increased proteolytic activity within the lesion
5.2.1. Matrix metalloproteinases
5.2.2. Cathepsins
5.3. Cell death/apoptosis
5.3.1. Macrophage death
5.3.2. Smooth muscle cell death
6. Diabetic animal models of plaque rupture
7. Factors affecting thrombus formation in the diabetic state
7.1. Increased thrombogenicity
7.2. Decreased fibrinolysis
8. Conclusions and perspective
9. Acknowledgments
10. References

1. ABSTRACT

Risk factors for atherosclerosis, including diabetes, have been identified for many years, and, especially for lipids, we now know a great deal about how these factors contribute to the early lesion. In contrast, the mechanism or mechanisms linking these factors to the final events in atherosclerosis, that is plaque rupture and thrombotic occlusion, have remained largely speculative. This speculation has been because of a lack the ability to visualize fragile, advanced lesions in humans and a lack of animal models. Recently, however, magnetic resonance imaging tools have begun to visualize the advanced lesion and animal models with features of plaque rupture have been characterized. Thus the opportunity exists to test hypotheses linking diabetes to events in the advanced plaques. This review summarizes our current knowledge of the processes that may be responsible for increased plaque rupture and occlusion associated with diabetes.

2. INTRODUCTION

People with diabetes have quantitatively more atherosclerosis, especially between 10-70 years of age than the general population (1). This is most likely explained by factors in the diabetic environment that act as "enhancers" of the effects of other atherosclerosis risk factors, such as hyperlipidemia, hypertension or smoking. While the relative roles of hyperglycemia or insulin resistance remain unclear, the combined diabetic syndrome results in an acceleration of atherosclerotic lesion initiation, progression, or both.

The diabetic syndrome also appears to be associated with events in the advanced lesion leading to patient death. Based on morphological studies of advanced lesions, these events appear to require disruption of the surface of the lesion, either by loss of the endothelium overlying the lesion, called erosion, or by rupture of the lesion with exposure of the necrotic core (2). In either case subsequent thrombus formation is the primary cause of sudden cardiac death. Both type 1 and type 2 diabetes are associated with increased cardiovascular mortality compared to the general population (3).

Presumably the morphological basis for this increased mortality in people with diabetes is due to an increased frequency of the malignant lesions described in

the general atherosclerotic population. Although there is surprisingly little morphological data specifically devoted to the atherosclerotic lesion in diabetes, an *in vivo* study, utilizing percutaneous coronary angioscopy in a small number of patients with unstable angina, shows that patients with type 2 diabetes have an increased frequency of ulcerated plaques (94% versus 60% of plaques in non-diabetic patients) and intracoronary thrombus formation (94% versus 55% in non-diabetic patients) (4). Since coronary risk factors, such as cholesterol levels, hypertension and smoking were similar between the two groups, the morphological data support the hypothesis that plaque rupture and thrombus formation are, as expected, enhanced by diabetes.

Even if more extensive data support this expected relationship between morphology and diabetes, it is important to keep an open mind about how diabetes might accelerate risk. Clinical risk, after all, could be related to enhanced thrombogenicity or even an altered susceptibility to arrhythmia rather than to lesion morphology. On the other hand, the diabetic syndrome might primarily effect lesion initiation since one assumes that the more rapidly early lesions form, the more likely there would be progression to clinically significant events.

3. INCREASED CARDIOVASCULAR MORTALITY IN PATIENTS WITH DIABETES

Several major clinical studies have investigated the effects of improved blood glucose, lipid abnormalities and hypertension on cardiovascular events and mortality associated with type 1 and type 2 diabetes.

3.1. What is the role of hyperglycemia?

Improved blood glucose control has been shown by both the Diabetes Control and Complications Trial (DCCT, 5) and the United Kingdom Prospective Diabetes Study (UKPDS, 6) to have no significant effect on cardiovascular complications in people with type 1 diabetes (5) or type 2 diabetes (6). Interestingly, microvascular complications were significantly reduced in both of these studies, showing a strong dependence of microvascular complications on blood glucose control (5-6). The number of cardiovascular events was small in the DCCT study (5), and several additional interventional studies are currently investigating the effects of intensified blood glucose control on cardiovascular disease in diabetes (7).

The DCCT and UKPDS studies are in seeming conflict with epidemiological studies that show that the increased risk of death among men with diabetes can be largely explained by elevated glycated hemoglobin A1c (HbA1c) levels (a marker of blood glucose control). In one large study, an increase of 1% in HbA1c was associated with a 28% increase in risk of death independent of age, blood pressure, serum cholesterol, body mass index, and cigarette smoking habit (8). One should bear in mind however, that the effects hyperglycemia are difficult to separate from other metabolic disturbances associated with diabetes, and that epidemiological studies cannot distinguish between a direct effect of hyperglycemia on cardiovascular mortality and effects of hyperglycemia-associated factors. For example, total fat intake has been shown be associated with levels of HbA1c in people without diabetes (9). Thus, at present it is unclear to what extent hyperglycemia directly contributes to cardiovascular mortality in diabetes.

3.2. What is the role of dyslipidemia?

Type 2 diabetes is associated with dyslipidemia, and dyslipidemia is considered the most significant cardiovascular risk factor in people with type 2 diabetes (10). The dyslipidemia is, in fact, often present before overt type 2 diabetes is evident, and is part of the metabolic syndrome, or syndrome X (11). The three major components of dyslipidemia associated with the metabolic syndrome and type 2 diabetes are increased fasting and postprandial triglyceride-rich particles, including very low-density lipoprotein (VLDL), decreased high-density lipoprotein (HDL), and increased small, dense low-density lipoprotein (LDL) particles (10, 12). Several interventional studies have shown that, when present, elevated lipid levels appear to contribute to a similar extent to cardiovascular disease in people with type 2 diabetes as in people without diabetes (13-16). These studies have also shown that control of dyslipidemia does not completely eliminate cardiovascular risk in patients with diabetes (17). Thus, lipid abnormalities, when present, clearly contribute to cardiovascular events in people with type 2 diabetes, but do not fully explain the increased cardiovascular risk.

People with type 1 diabetes usually have less severe dyslipidemia compared to people with type 2 diabetes. However, poorly controlled type 1 diabetes is associated with elevated plasma cholesterol, and triglycerides are frequently elevated (18). Even in well-regulated patients without micro- and macrovascular complications, lipoprotein composition abnormalities are often present (19-20). However, the strongest predictor of cardiovascular mortality in people with type 1 diabetes is diabetic nephropathy (21). With the development of diabetic nephropathy the cardiovascular risk increases markedly, and nephropathy is associated with increased concentrations of cholesterol and low levels of HDL (22). Clinical intervention studies are needed to investigate the effects of lipid therapy on cardiovascular mortality in patients with type 1 diabetes.

3.3. What is the role of hypertension?

The effect of hypertension and the renin-angiotensin system on cardiovascular disease in people with type 1 and type 2 diabetes was recently studied in the large Heart Outcomes Prevention Evaluation (HOPE) trial. The results from this study show that ramipril, an angiotensin-converting-enzyme (ACE) inhibitor, caused a significant reduction in death from cardiovascular disease in people with diabetes. However, the benefit was not greater in people with diabetes than in people with other risk factors, and did not appear to be due to reduction of blood pressure (23). As with hyperlipidemia, hypertension appears to contribute a similar extent to cardiovascular disease in people with diabetes as in people without diabetes (13, 16). Thus, the renin-angiotensin system and

hypertension contribute significantly to cardiovascular events both in people with and without type 1 and type 2 diabetes.

4. HOW DOES DIABETES AFFECT LESION FORMATION AND PROGRESSION?

4.1. Theories of lesion formation and progression

4.1.1. Lesion initiation

It is generally believed that infiltration of monocytes into the subendothelial space and subsequent accumulation of lipid-loaded macrophages initiate formation of lesions of atherosclerosis. In young humans, accumulation of lipid-loaded macrophages is often seen in areas with intimal thickening, called intimal cell masses, that form in the subendothelial space in locations likely to lead to frank atherosclerosis in the adult (2, 24). This suggests that products of these smooth muscle cells may be critical to lesion localization (25). In many small animal models, on the other hand, infiltration of monocytes and subsequent activation and differentiation of these cells into lipid-loaded macrophages is often seen in areas without preexisting intimal thickening. Since these macrophage foam cell masses, i.e. xanthomas, occur at sites in young humans that do not show lesions in adults, it has been suggested that the xanthomatous lesions regress unless a fibrous cap of smooth muscle is formed or exists to encapsulate the fatty mass (2).

Over the last decade, a consensus has developed that the early formation of a fatty lesion and its progression to an encapsulated lesion is the result of an inflammatory process (26-27). There is evidence, moreover, that the lesion contains oxidized LDL as an inflammatory stimulus. This view is supported by the observation of antibodies to oxidized epitopes in the blood of mice with apolipoprotein E (apoE)-/- atherosclerosis (28) and observation of T cells that recognize oxidized LDL epitopes in human lesions (29). T cells can enhance atherogenesis for example by secreting interferon-gamma, a cytokine that activates macrophages. It has been shown that the interferon-gamma producing subset of T cells (Th1 cells) is present in lesions of atherosclerosis (30). Recently a specific mutation, the deletion of 5-lipoxygenase, has been identified as blocking all these initial events, but the mechanism of action of 5-lipoxygenase in this process is as yet unknown (31).

Diabetes appears to enhance foam cell lesion formation in experimental animals and in humans. In animal models, type 1 diabetes induced by toxins, alloxan or streptozotocin, or by autoimmune-mediated beta cell destruction increases formation of fatty streak lesions (32-34). There is similar evidence from human postmortem studies that diabetes accelerates lesion initiation defined as the presence of fatty streaks. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study investigated atherosclerosis in ~3000 youths, 15-34 years of age, who died of trauma. Although the number of subjects with diabetes was small, the study showed that youths over 25 years of age with elevated levels of glycated hemoglobin (> 8%), had significantly more fatty streaks in the right coronary artery than controls (35). The PDAY study also showed that youths 15-24 years of age with favorable lipid profiles and elevated levels of glycated hemoglobin had significantly more fatty streaks in the right coronary artery than youths without elevated glycated hemoglobin (36). A recent high-resolution ultrasound *in vivo* study of common carotid arteries of 11-year old children with type 1 diabetes showed that these children had an increased intima-media thickness compared to a matched control group (37). The increased intima-media thickness in children with type 1 diabetes did not appear to be due to conventional risk factors, such as blood pressure, total cholesterol, triglycerides, LDL or HDL levels (37). Together, these results indicate that diabetes, in the absence of conventional risk factors, accelerates lesion initiation in humans.

4.1.2. Lesion progression

Two steps characterize the conversion of the xanthomatous lesions to first, the classical lesion described by Virchow in 1855 (38) and then to advanced, clinically significant lesions. These steps are the massive death of macrophages to form a necrotic core and the encapsulation of the macrophage-rich mass by smooth muscle cells (SMCs) to form a fibrous cap. The temporal and causal relationship between these events is not known although studies using platelet-derived growth factor (PDGF) antagonists to block fibrous cap formation suggest, surprisingly, that the SMCs may enhance formation of the necrotic core (39-40). Very little is known about the processes underlying these changes. Speculation about the role of specific growth factors, including PDGF, in the formation of the fibrous cap is only partially supported by experiments in animal models (39-40). Similarly, there is speculation but not hard evidence that oxidized LDL plays a critical role in death of the plaque macrophage (41-42).

The consensus is that ultimately the lesion is destabilized by thinning of the SMC-rich fibrous cap, increased macrophage content and increased proteolytic activity within the lesion, leading to digestion of the connective tissue of the cap (2, 43-45). How may inflammation directly contribute to plaque rupture? The consensus hypothesis is that pro-inflammatory mediators, such as interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) released from macrophages in the lesion can stimulate expression of matrix metalloproteinases (MMPs). Increased MMP levels would then promote degradation of collagens and weaken the strength of the fibrous cap (44, 46-47). So far, this hypothesis has not been directly tested in animals. The studies that have been done so far, either overexpressing MMP1 in macrophages in apoE-deficient mice (48), or knocking out the endogenous MMP inhibitor TIMP-1 (49), have not found signs of plaque rupture or thrombosis. However, these studies could not distinguish between effects of MMPs on lesion initiation and progression, because the transgene was present from birth. Other studies in mice have shown that inflammatory mediators, such as monocyte chemoattractant protein-1 (MCP-1) and CD40 lead to an increased macrophage content and decreased SMC and collagen content (50-54). Again, these

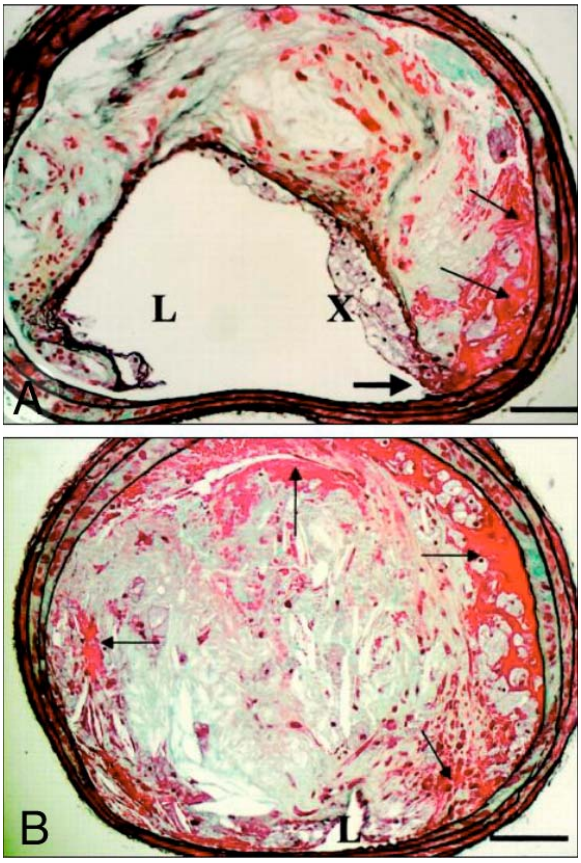

Figure 1. Intra-plaque hemorrhage in advanced lesions in brachiocephalic arteries from apoE-deficient mice. A. An example of lesion with intra-plaque hemorrhage in brachiocephalic artery from a 42-week-old apoE-deficient mouse is shown. The section was stained with a modified Movat's pentachrome stain. The hemorrhage (thin arrows) appears to be associated with the lateral xanthoma, and a rupture or fissure occurred at the extreme lateral margin (thick arrow). X indicates xanthoma. L indicates lumen. Bar=100 microm. B. Hemorrhage in stenotic lesion in innominate artery of apoE-/- mouse. An almost totally occluded innominate artery from a 54-week-old apoE-/- mouse is shown. The lesion contains several areas of intraplaque hemorrhage (arrows). The source of the hemorrhage is not apparent but may have been a xanthomatous region, as indicated by the number of intact foam cells within the major area of hemorrhage visible in this section. Bar=100 microm. Published by permission of Lippincott, Williams & Wilkins, from Rosenfeld ME, Polinsky P, Virmani R, Kauser K, Rubanyi G, Schwartz SM. Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse (55).

changes occur in macrophage rich lesions that do not rupture so the causal connection with lesions that do rupture remains to be proven.

An alternative hypothesis has arisen recently because of evidence in apoE -/- mice that lesions spontaneously rupture by death of macrophages located at the shoulder regions of carotid plaques (55). Destabilization of the lesion is believed to cause rupture or fissuring of the shoulder regions, and subsequent thrombus formation. Again, in the mouse, animals fed a very lipid rich diet can die spontaneously due to occlusive thrombosis overlying ruptured carotid lesions (56). Whether such macrophage-rich lateral lesions exist in humans is not known.

The emphasis on plaque rupture as a final event is compromised by clear evidence that occlusion can occur without rupture, especially in younger patients. Virmani and colleagues have shown that thrombotic occlusion of a coronary artery can occur without evidence of rupture or even the presence of a necrotic core. This poorly understood process seems to be associated with loss of the endothelium and has, therefore, been termed erosion (43).

Just as the processes leading to plaque rupture and thrombotic occlusion may be partly distinct, there is ample evidence that the processes causing lesion initiation and lesion progression are distinct. For example, vitamin C-deficiency does not influence the initiation of lesions but induces plaques that appear vulnerable to rupture (57). The PDGF beta receptor and CD40 ligand are more important in lesion progression than in initiation (40, 50). Blockade of the colony stimulating factor (CSF) receptor c-fms, on the other hand, has been shown to inhibit lesion initiation without affecting the size of advanced lesions (58). Similarly, estrogen has recently been shown to inhibit lesion initiation but to have no effect on intra-plaque hemorrhage and progression of established lesions in apoE-deficient mice (59). Thus, lesion initiation and progression are separable events.

To date, there is no evidence that diabetes directly affects lesion progression. That is, the increased cardiovascular mortality associated with diabetes may be due solely to an accelerated lesion initiation, or to stimulatory effects of diabetes on both lesion initiation and progression.

4.2. Non-diabetic animal models of plaque rupture and occlusion

Detailed studies of the mechanisms leading to plaque rupture and occlusion are not possible without relevant animal models. Most animal models used to date, e.g. quails, rabbits, hamsters, pigs, and non-human primates, develop fatty streak lesions and atheromas, but plaque rupture and occlusion are rare or absent. Recently, studies on the mechanisms leading to plaque rupture have become much more feasible due to the characterization of mouse models that exhibit features of plaque rupture (60-61). In both apoE-deficient and LDL receptor (LDLR)-deficient mice, the brachiocephalic artery can form unstable lesions in old mice (55-56, 62-64), as shown in figure 1. The brachiocephalic artery (innominate artery) is the first branch from the aortic arch, bifurcating to form the right common carotid artery and the right subclavian artery. The unstable lesions formed at this site are characterized by fibrous cap thinning, intra-plaque hemorrhage and disruption of the fibrous cap as a result of macrophage death. Intra-plaque hemorrhage can only occur during life,

and therefore intra-plaque hemorrhage is a strong indication that the lesion has ruptured in the live animal.

Intramural thrombus formation is usually extremely rare in mouse models. In a recent study, ~3 % of apoE-deficient or LDLR-deficient mice fed a cholesterol-rich diet for up to 12 months developed aortic thrombi (62). In another study, apoE-deficient mice on a C57BL6/129SvJ background were fed a cholesterol-rich diet for up to 14 months (64). Of the 98 mice studied, 52% had ruptured plaques in the brachiocephalic artery, and 73% of mice with ruptured plaques showed signs of luminal thrombosis (64). Some animals also showed myocardial infarctions (64). Unfortunately, there appear to be marked strain differences in the susceptibility of mice to plaque rupture. In the study by Williams *et al.* (64), apoE-deficient mice on a 50% C57BL6, 50% 129SvJ background demonstrated extensive plaque rupture, thrombus formation and sudden death, whereas thrombus formation and sudden death are not observed in apoE-deficient mice on a nearly pure (10 generation back-cross) C57BL6 background. The difference may be explained, in part, by the fact that 129Sv mice absorb dietary cholesterol more efficiently than C57BL6 mice do (65). Finally, rupture-associated thrombosis has also been seen in a few apoE-knockout mice in which a silastic collar was placed around to carotid artery to induce plaques, the p53 tumor suppressor was overexpressed by means of an adenoviral vector and the mice were made hypertensive with phenylephrine (66). Together, these results show that plaque rupture, defined as intra-plaque hemorrhage and ruptures of the plaque, is common in old apoE-deficient or LDLR-deficient mice but that intraluminal thrombosis is much more rare. Thus, these models should be useful in the study of the mechanisms leading to plaque rupture, but are less useful for studies of events leading to arterial occlusion caused by thrombosis.

5. WHAT CELLULAR EVENTS ARE LIKELY TO BE INVOLVED IN PLAQUE RUPTURE AND EROSION AND HOW DOES DIABETES AFFECT THESE EVENTS?

5.1. Inflammation

Inflammation plays a key role in initiation of atherosclerosis, and most likely also in the processes leading to plaque rupture and arterial occlusion (26). In large clinical studies, increased plasma levels of C-reactive protein (CRP) have been shown to serve as a strong marker for risk of coronary events (67). CRP is an acute phase reactant produced by the liver in response to inflammatory cytokines. Although there is no evidence that CRP itself has a direct role in the pathogenesis of plaque rupture, these findings show a strong correlation between inflammation and final cardiovascular events.

Diabetes most likely results in increased inflammation in advanced plaques. For example, it has been shown that atherectomy specimens from diabetic patients undergoing coronary atherectomy for symptomatic coronary artery disease have a larger content of macrophages than specimens from patients without diabetes (68). One theory is that increased levels of modified LDL could explain increased inflammatory and immune reactions in diabetes. Glycation of LDL under hyperglycemic conditions is likely to result in increased formation of oxidized LDL (69). Both oxidized LDL and glycated LDL also induce formation of immune complexes, which, at least *in vitro*, exert pro-inflammatory effects by stimulating cytokine release from macrophages (70). Other studies show that blockade of the receptor for advanced glycation endproducts (RAGE) results in decreased inflammation in pre-formed lesions in streptozotocin-diabetic apoE-deficient mice (71). Because RAGE binds to a number of interesting ligands, in addition to advanced glycation endproducts (AGEs), it is not known if the effects of RAGE blockade in this study were due to AGEs. In fact, RAGE blockade reduced atherosclerotic lesion size (71) and intimal thickening after arterial injury (72) in non-diabetic mice, suggesting that the role for RAGE ligands is not dependent on the diabetic state.

Perhaps the most commonly believed theory is that diabetes results in increased expression of molecules that promote monocyte adherence to the endothelium and subsequent migration into the subendothelial space. This theory is supported by ample animal and *in vitro* studies, and some human studies. Thus, vascular cell adhesion molecule-1 (VCAM-1) expression is stimulated by high glucose levels and AGEs in endothelial cells in culture (73-74), in animals (74-75) and in patients with diabetes (76). Furthermore, lesions in human coronary arteries from patients with diabetes have been shown to exhibit increased levels of immunoreactive fractalkine, a chemokine that mediates firm adhesion of leukocytes, compared to lesions from non-diabetic patients (77). Taken together, these studies show that diabetes may increase inflammation in advanced lesions of atherosclerosis and it is reasonable to posit that such changes would accelerate plaque rupture.

5.2. Increased proteolytic activity within the lesion

5.2.1. Matrix metalloproteinases

Another hypothesis is that plaque rupture may be caused by an increased proteolytic activity within the lesion that would promote extracellular matrix degradation and thereby weakening of the fibrous cap (26, 78). The enzymes believed to be involved in this process are the MMPs and the cathepsins (discussed below). MMPs are endopeptidases that degrade a variety of extracellular matrix components and other proteins. Several MMPs have been characterized: MMP-1 (interstitial collagenase), MMP-2 (72-kDa gelatinase, gelatinase A), MMP-3 (stromelysin-1), MMP-7 (matrilysin), MMP-9 (92-kDa gelatinase, gelatinase B), MMP-12 (metalloelastase), MMP-13 (interstitial collagenase-3) and MMP-14 (membrane type 1-MMP). MMPs are secreted from different cell types within the lesion as pro-enzymes, and enzymatic activation involves removal of the pro-domain. This can be mediated by proteases, such as membrane-type MMPs (MT-MMPs), by plasmin, or by autolysis (78). MMP activity is also regulated by the endogenous inhibitors TIMP-1 and TIMP-2. As discussed above, there is not yet direct evidence that MMPs play a role in plaque rupture or erosion. The main result supporting this theory

is that MMP expression and activity have been demonstrated in the vulnerable shoulders of human atheromas (26, 46, 79). Animal studies on overexpression of different MMPs in advanced plaques will most likely show whether MMPs indeed play a role in plaque rupture.

A few studies have investigated MMP levels and activities in the diabetic setting. Serum levels of MMP-2, MMP-8 and MMP-9 are increased in patients with type 2 diabetes (80) and MMP-9 levels and activity are increased in aortas from streptozotocin-diabetic rats compared to controls (81). On the other hand, MMP-1, MMP-2 and MMP-9 levels, as well as MMP activity, have been found to be decreased in internal mammary artery specimens from patients with diabetes (82). Since this artery is usually protected from atherosclerosis, these findings do not necessarily mean that levels of MMPs are decreased in advanced plaques from diabetic patients. This is an area that requires further studies.

5.2.2. Cathepsins

It has been speculated that another group of proteases, the cathepsins, may be involved in causing plaque rupture. The cathepsins are cysteine proteinases that degrade elastin and fibrillar collagen. Cathepsins K, L and S all have potent elastolytic activity. Cathepsin S is the most potent elastase known (83), whereas cathepsin K cleaves collagen I and III (84). The cathepsins were originally thought to function only in acidic lysosomes, but recent research shows that they can be released by macrophages (85). Macrophages in human lesions contain abundant immunoreactive cathepsin K and S (86), and atherosclerotic lesions from apoE-deficient mice express cathepsins B, D, L and S (87). Cathepsin B has even been suggested as a biomarker of vulnerable plaques (88). Expression levels of cathepsins C and D are up-regulated in aneurysms (89), which also contain lower levels of the endogenous cysteine protease inhibitor cystatin C (90). Furthermore, human SMCs in culture can be stimulated to secrete active cathepsin S by IL-1beta or interferon-gamma (86). Together, these findings suggest that the cathepsins may be involved in causing plaque rupture or aneurysms. So far, the presence of cathepsins and cystatin C in lesions from patients with diabetes has not been studied.

5.3. Cell death/apoptosis

The third major hypothesis on the cellular events causing plaque rupture states that increased cell death, by necrosis and/or apoptosis, is responsible for rupture. Traditionally, cell death by apoptosis and necrosis have been considered two separate events with very different outcomes. Whereas apoptotic cell death has been believed to be "silent", without release of cellular components to the extracellular space, necrotic cell death has been characterized by a loss of cell membrane integrity and leakage of lysosomal enzymes associated with inflammation. However, recent studies have shown that there is a great deal of overlap in the morphological and biochemical events in cells undergoing apoptosis and necrosis. For example, the dogma that apoptotic cell death is “silent” has recently been challenged by studies that clearly show a release of cytokines and induction of an inflammatory response in the arterial wall following apoptosis induced by overexpression of Fas-associating death domain protein (FADD), one of the signaling molecules in the apoptotic pathway (91). For these reasons, the boundary between apoptosis and necrosis is not well defined. In this review, we will therefore refer to cell death rather than to classify the type of death as apoptotic or necrotic.

5.3.1. Macrophage death

As already discussed, a critical feature of the change from the macrophage foam cell lesion, more properly called a xanthoma, to a true atheroma is the formation of a necrotic core (2). Formation of a necrotic core is due to macrophage death. The mechanisms underlying this death have only recently begun to be studied. One of the factors that cause macrophage death is free cholesterol (41). Acyl coenzyme A:cholesterol acyltransferase (ACAT) catalyzes the formation of cholesterol esters from free cholesterol. Accordingly, LDLR-deficient mice that received macrophages deficient in ACAT had lesions characterized by increased levels of free cholesterol and increased macrophage death as compared to mice that received wild type macrophages (92). No evidence of plaque rupture was observed in these mice, perhaps because only an early time point (12 weeks on a fat-enriched diet) was studied.

A very different hypothesis has emerged from the study of differences of the effects of oxidized LDL on normal murine macrophages versus macrophages from mice lacking a functional Fas receptor. The normal cells die massively after exposure to oxidized LDL (30 microg/ml), with nearly 100% death by 4 days (93). In contrast, the dysfunctional Fas cells show little or no death under this circumstance. Moreover the wild type cells but not the Fas-defective cells show spontaneous activation of caspases even before exposure to oxidized LDL. These data are reminiscent of the massive macrophage death seen in atherosclerotic cores and suggest that the process may be inhibited by an absence of Fas. A recent preliminary study has directly addressed the role of increased macrophage death in atherogenesis. In this study, LDLR-deficient mice received macrophages from Fas-deficient lpr mice and where then fed an atherogenic diet for 16 weeks (94). Although the extent of atherosclerosis was similar in the two groups of mice, the Fas-deficient bone marrow transplant resulted in less TUNEL staining in the lesions (indicative of less apoptosis), a larger lipid core and a thinner fibrous cap (94). These studies do not, however, address the role of macrophage death in plaque rupture because the Fas-deficient macrophages were introduced prior to fat feeding. It would be very interesting to investigate if plaque rupture could be prevented by introducing the Fas-deficient macrophages at a time when advanced lesions are formed.

5.3.2. Smooth muscle cell death

Since a thin fibrous cap and low number of SMCs versus macrophages are often seen in ruptured lesions, it has been proposed that loss of SMCs in the cap of fibroatheromas may contribute to plaque rupture. SMC

death may lead to plaque instability because most of the interstitial collagen fibers, which are important for the strength of the fibrous cap, are produced by SMCs (95-96). This hypothesis is based, so far, on correlative studies. Several studies have shown an increased SMC apoptosis, measured as TUNEL-positive cells, in advanced lesions (95, 97-98). Conversely, it has been shown that treatments that prevent, or lead to regression of cardiovascular disease, cause an increased SMC content, a decreased macrophage content (99-100), and an increased collagen content (101) in lesions. Interestingly, it has been demonstrated that the SMCs derived from atherosclerotic plaques are more sensitive to inducers of apoptosis than SMCs derived from normal vessels (102-103). This may be due to differential expression of pro- and anti-apoptotic molecules in SMCs derived from plaques versus SMCs derived from the media. Despite the correlation between loss of SMCs in the fibrous cap and plaque rupture, direct evidence that SMC death indeed causes plaque rupture is still missing.

The effects of diabetes on SMC apoptosis are unclear. It has been shown that streptozotocin-diabetic rats without atherosclerosis have an increased number of TUNEL-positive aortic SMCs compared to non-diabetic rats (104), but that hyperglycemia protects against medial SMC death in mice (105). In cultured SMCs, elevated levels of glucose have been found to either stimulate death (106), or prevent cell death (105, 107-108). It is not known if diabetes affects SMC death in advanced lesions of atherosclerosis.

6. DIABETIC ANIMAL MODELS OF PLAQUE RUPTURE

If the number of models available to study plaque rupture in non-diabetic animals is small, even fewer models are currently available to study plaque rupture in the diabetic setting. However, in a recently developed porcine model, steptozotocin-induced diabetes in combination with fat feeding was found to cause advanced lesions in male Yorkshire swine whereas lesions in non-diabetic fat-fed swine were not as advanced (109). In the right and left coronary arteries, these advanced lesions had signs of necrosis, calcification and intra-plaque hemorrhage. In the pig, intra-plaque hemorrhage may be due to plaque rupture or possibly to the presence of intra-plaque vessels (mice lack intra-plaque vessels; Schwartz *et al.* unpublished observation). Therefore, it is possible that the intra-plaque hemorrhage seen in this pig model represents plaque rupture, but the contribution of intra-plaque vessels has not yet been ruled out. The formation of advanced plaques occurred concomitantly with hyperglycemia (approximately 6 mM in non-diabetic animals and 18 mM in diabetic animals) and elevated levels of triglycerides (approximately 0.25 mM in non-diabetic animals and 0.65 mM in diabetic animals). These observations indicate that diabetes may accelerate plaque rupture by increasing blood glucose levels, by increasing triglyceride levels, or by other unidentified factors.

We have recently developed a new mouse model of diabetes-accelerated atherosclerosis (33). LDLR-deficient mice were crossed with a transgenic mouse model of autoimmune type 1 diabetes (both on a C57BL6 background) used previously to study the mechanisms involved in beta cell loss, the RIP-LCMV-GP+ mouse (110). In this transgenic mouse, the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) is expressed under control of the rat insulin promoter (RIP) on pancreatic beta cells. The GP is seen by the immune system as an auto-antigen, and its presence does not cause insulitis or diabetes (110). Instead, the significance of the expressed GP is that when the mouse is infected with LCMV, it develops an autoimmune attack on the beta cells very similar to the process involved in induction of type 1 diabetes in humans (111). In diabetic RIP-LCMV-GP;LDLR-/- mice fed a cholesterol-rich diet for 12 weeks, intra-plaque hemorrhage appeared frequent in the brachiocephalic artery, whereas intra-plaque hemorrhage was rare in non-diabetic controls (Bornfeldt *et al*., unpublished preliminary results). It is important to point out that neither the diabetic swine model (109) nor the RIP-LCMV-GP;LDLR-/- diabetic mouse model (33) show signs of luminal thrombosis at the time-points studied. It is possible that extended time points would reveal such events, but the urgent need to develop animal models of diabetes-associated arterial occlusion remains.

7. FACTORS AFFECTING THROMBUS FORMATION IN THE DIABETIC STATE

Although our focus so far has been on the advanced lesion, it is equally important to consider the role of systemic factors in increasing the probability of cardiovascular death. Alterations in systemic factors could cause increased cardiovascular death even if there were no differences in the extent of advanced lesions.

7.1. Increased thrombogenicity

A large body of evidence indicates that diabetes is associated with a pro-thrombotic state, which is likely to promote arterial occlusion initiated by plaque rupture or erosion. It is possible that this increased thrombogenicity can explain the increased cardiovascular mortality in patients with diabetes. Thus, diabetes is associated with increased platelet aggregation (112-119), plasma fibrinogen levels (118, 120), and pro-coagulant factor VIII (112, 118, 121-122).

7.2. Decreased fibrinolysis

Diabetes and insulin resistance also appear to cause decreased endogenous fibrinolysis (118, 120, 122-123). One mechanism may be through an upregulation of plasminogen activator inhibitor-1, PAI-1 (123-125). Interestingly, a recent study reported spontaneous coronary arterial thrombosis in transgenic mice expressing a stable form of human PAI-1 (126). Because these mice were not apoE-deficient or LDLR-deficient, they have no atherosclerotic lesions, and are therefore not a model of plaque rupture. No animal models are currently available to study the mechanisms leading to thrombosis in diabetes, and such models are urgently needed.

8. CONCLUSIONS AND PERSPECTIVE

Understanding the mechanisms that cause plaque rupture and arterial occlusion in diabetes is clearly very important, both clinically and scientifically. The recent

characterization of murine models that show features of plaque rupture greatly increases the feasibility of studies of the cellular and molecular events involved in plaque rupture in both diabetic and non-diabetic animals. Similarly, although it is beyond the scope of this review, the development of MRI methods that can visualize the fibrous cap and evaluate extents of lesion instability in human vessels without invasion (127-130), open the possibility that we may be able to learn a lot more about the effects of the diabetic syndrome on lesions in humans.

Several important questions need to be addressed. First, it is not known if diabetes accelerates only initiation of lesions of atherosclerosis or if factors associated with diabetes directly stimulate the processes involved in plaque rupture and occlusion, nor are these factors known. Secondly, in will be critical to determine if hyperglycemia and/or dyslipidemia associated with type 1 and type 2 diabetes cause plaque rupture. Thirdly, with the generation of several very interesting knockout mouse models, it is now feasible to start asking questions about the role of specific molecules, such as proteinases or Fas, in plaque rupture in diabetes. The next few years are likely to see significant increase in our knowledge about the mechanisms leading to plaque rupture and arterial occlusion in diabetes.

9. ACKNOWLEDGMENTS

The work by senior fellows, students and technical personnel in the authors' laboratories is gratefully acknowledged. This work was supported in part by grants from the NIH, HL62887, the Juvenile Diabetes Research Foundation, 1-2003-33, an Innovation Award from the American Diabetes Association and by the Royalty Research Fund at the University of Washington to Dr. Bornfeldt and by NIH grants HL58083 and HL072262 to Dr. Schwartz.

10. REFERENCES

1. Ferrier T.M: Comparative study of arterial disease in amputated lower limbs from diabetics and non-diabetics (with special reference to feet arteries). *Med J Aust* 1, 5-11 (1967)

2. Virmani R, F.D. Kolodgie, A.P. Burke, A. Farb & S.M. Schwartz: Lessons from sudden coronary death. *Arterioscler Thromb Vasc Biol* 20, 1262-1275 (2000)

3. Ruderman N.B. & C. Haudenschild: Diabetes as an atherogenic factor. *Prog Cardiovasc Dis* 26, 373-412 (1984)

4. Silva J.A, A. Escobar, T.J. Collins, S.R. Ramee & C.J. White: Unstable angina. A comparison of angioscopic findings between diabetic and nondiabetic patients. *Circulation* 92, 1731-1736 (1995)

5. DCCT; The Diabetes Control and Complications Trial Research Group: The effects of intensive treatment of diabetes on the development and progression of long-term complications of insulin-dependent diabetes mellitus. *N Engl J Med* 329, 977-986 (1993)

6. UKPDS Group: Intensive blood-glucsoe control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS33). *Lancet* 352, 837-853 (1998)

7. Duckworth W.C: Hyperglycemia and cardiovascular disease. *Curr Atheroscler Rep* 3, 383-391 (2001)

8. Khaw K.T, N. Wareham, R. Luben, S. Bingham, S. Oakes, A. Welch & N. Day: Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of european prospective investigation of cancer and nutrition (EPIC-Norfolk). *BMJ* 322, 15-18 (2001)

9. Harding A.H, L.A. Sargeant, A. Welch, S. Oakes, R.N. Luben, S. Bingham, N.E. Day, K.T. Khaw & N.J. Wareham: Fat consumption and HbA(1c) levels: the EPIC-Norfolk study. *Diabetes Care* 24, 1911-6 (2001)

10. Taskinen M.R: Diabetic dyslipidemia. *Atheroscler* Suppl 3, 47-51 (2002)

11. Reaven G.M: Multiple CHD risk factors in type 2 diabetes: beyond hyperglycaemia. *Diabetes Obes Metab* 4 Suppl 1, S13-S18 (2002)

12. Ruotolo G. & B.V. Howard: Dyslipidemia of the metabolic syndrome. *Curr Cardiol Rep* 4, 494-500 (2002)

13. Yki-Järvinen H: Management of type 2 diabetes mellitus and cardiovascular risk. *Drugs* 60, 975-983 (2001)

14. Steiner G: Treating lipid abnormalities in patients with type 2 diabetes mellitus. *Am J Cardiol* 88, 12 Suppl 1, 37-40 (2001)

15. Robins S.J: Targeting low high-density lipoprotein cholesterol for therapy: lessons from the Veterans Affairs High-density Lipoprotein Intervention Trial. *Am J Cardiol* 88, 12 Suppl 1, 19-23 (2001)

16. Huang E.S, J.B. Meigs & D.E. Singer: The effect of interventions to prevent cardiovascular disease in patients with type 2 diabetes mellitus. *Am J Med* 111, 633-642 (2001)

17. Gotto A.M: Lipid management in diabetic patients: lessons from prevention trials. *Am J Med* 112 Suppl 8A, 19S-26S (2002)

18. Ginsberg H.N: Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis. *Diabetes Care* 14, 839-55 (1991)

19. Dunn F.L: Plasma lipid and lipoprotein disorders in IDDM. *Diabetes* 41, Suppl 2,102-106 (1992)

20. Garg A: Management of dyslipidemia in IDDM patients. *Diabetes Care* 17, 224-234 (1994)

21. Weinstock Brown W. & W.F. Keane: Proteinuria and cardiovascular disease. *Am J Kidney Dis* 38, 4 Suppl 1, S8-S13 (2001)

22. Dullaart R.P: Plasma lipoprotein abnormalities in type 1 (insulin-dependent) diabetes mellitus. *Neth J Med* 46, 44-54 (1995)

23. HOPE study investigators: Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* 355, 253-259 (2000)

24. Stary H.C, A.B. Chandler, R.E. Dinsmore, V. Fuster, S. Glagov, W.Jr. Insull, M.E. Rosenfeld, C.J. Schwartz, W.D. Wagner & R.W. Wissler: A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council of Atherosclerosis, American Heart Association. *Circulation* 92, 1355-1374 (1995)

25. Williams K.J. & I. Tabas: The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol* 15, 551-561 (1995)

26. Libby P: Inflammation in atherosclerosis. *Nature* 420, 868-874 (2002)

27. Glass C.K. & J.L. Witztum: Atherosclerosis. The road ahead. *Cell* 104, 503-516 (2001)

28. Pratico D, R.K. Tangirala, S. Horkko, J.L. Witztum, W. Palinski & G.A. FitzGerald: Circulating autoantibodies to oxidized cardiolipin correlate with isoprostane F(2alpha)-VI levels and the extent of atherosclerosis in ApoE-deficient mice: modulation by vitamin E. *Blood* 97, 459-464 (2001)

29. Hansson G.K, P. Libby, U. Schonbeck & Z.Q. Yan: Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res* 91, 281-291 (2002)

30. Zhou X, G. Paulsson, S. Stemme & G.K. Hansson: Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. *J Clin Invest* 101, 1717-1725 (1998)

31. Mehrabian M, H. Allayee, J. Wong, W. Shih, X.P. Wang, Z. Shaposhnik, C.D. Funk & A.J. Lusis: Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res* 91, 120-126 (2002)

32. Dixon J.L, J.D. Stoops, J.L. Parker, M.H. Laughlin, G.A. Weisman & M. Sturek: Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. *Arterioscler Thromb Vasc Biol* 19, 2981-2992 (1999)

33. Renard C.B, L.A. Suzuki, F. Kramer, L.R. Tannock, M.G. von Herrath, A. Chait & K.E. Bornfeldt: A new murine model of diabetes-accelerated atherosclerosis. *Diabetes* 51, suppl. 2, 724 (2002)

34. Simionescu M, D. Popov, A. Sima, M. Hasu, G. Costache, S. Faitar, A. Vulpanovici, C. Stancu, D. Stern & N. Simionescu: Pathobiochemistry of combined diabetes and atherosclerosis studied on a novel animal model. The hyperlipemic-hyperglycemic hamster. *Am J Pathol* 148, 997-1014 (1996)

35. McGill H.C.Jr, C.A. McMahan, G.T. Malcom, M.C. Oalmann & J.P. Strong: Relation of glycohemoglobin and adiposity to atherosclerosis in youth. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Arterioscler Thromb Vasc Biol* 15, 431-440 (1995)

36. McGill H.C.Jr, C.A. McMahan, A.W. Zieske, G.T. Malcom, R.E. Tracy & J.P. Strong: Effects of nonlipid risk factors on atherosclerosis in youth with a favorable lipoprotein profile. *Circulation* 103, 1546-1550 (2001)

37. Järvisalo M.J, A. Putto-Laurila, L. Jartti, T. Lehtimäki, T. Solakivi, T. Rönnemaa & O.T. Raitakari: Carotid artery intima-media thickness in children with type 1 diabetes. *Diabetes* 51, 493-498 (2002)

38. Virchow R: Cellular Pathology as based upon physiological and pathological histology. *Classics of Medicine Library*, Birmingham, Alabama (1855)

39. Kozaki K, W.E. Kaminski, J. Tang, S. Hollenbach, P. Lindahl, C. Sullivan, J.C. Yu, K. Abe, P.J. Martin, R. Ross, C. Betsholtz, N.A. Giese, & E.W. Raines: Blockade of platelet-derived growth factor or its receptors transiently delays but does not prevent fibrous cap formation in ApoE null mice. *Am J Pathol* 161, 1395-1407 (2002)

40. Sano H, T. Sudo, M. Yokode, T. Murayama, H. Kataoka, N. Takakura, S. Nishikawa, S.I. Nishikawa & T. Kita: Functional blockade of platelet-derived growth factor receptor-beta but not of receptor-alpha prevents vascular smooth muscle cell accumulation in fibrous cap lesions in apolipoprotein E-deficient mice. *Circulation* 103, 2955-2960 (2001)

41. Yao P.M. & I. Tabas: Free cholesterol loading of macrophages induces apoptosis involving the Fas pathway. *J Biol Chem* 275, 23807-23813 (2000)

42. Yao P.M. & I. Tabas: Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. *J Biol Chem* 276, 42468-42476 (2001)

43. Virmani R, A.P. Burke, A. Farb & F.D. Kolodgie: Pathology of the unstable plaque. *Prog Cardiovasc Dis* 44, 349-356 (2002)

44. Libby P: Inflammation in atherosclerosis. *Nature* 420, 868-874 (2002)

45. Davies M.J: Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. *Circulation* 94, 2013-2020 (1996)

46. Galis Z.S, G.K. Sukhova, M.W. Lark & P. Libby: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 94, 2493-2503 (1994)

47. Saren P, H.G. Welgus & P.T. Kovanen: TNF-alpha and IL-1beta selectively induce expression of 92-kDa gelatinase by human macrophages. *J Immunol* 157, 4159-4165 (1996)

48. Lemaître V, T.K. O'Byrne, A.C. Borczuk, Y. Okada, A.R. Tall & J. D'Armiento: ApoE knockout mice expressing human matrix metalloproteinase-1 in macrophages have less advanced atherosclerosis. *J Clin Invest* 107, 1227-1234 (2001)

49. Lemaître V, Soloway PD, D'Armiento J. Increased medial degradation with pseudo-aneurysm formation in apolipoprotein E-knockout mice deficient in tissue inhibitor of metalloproteinases-1. *Circulation* 107, 333-338 (2003)

50. Lutgens E, K.B. Cleutjens, S. Heeneman, V.E. Koteliansky, L.C. Burkly & M.J. Daemen: Both early and delayed anti-CD40L antibody treatment induces a stable plaque phenotype. *Proc Natl Acad Sci USA* 97, 7464-7469 (2000)

51. Schonbeck U, G.K. Sukhova, K. Shimizu, F. Mach & P. Libby: Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. *Proc Natl Acad Sci USA* 97, 7458-7463 (2000)

52. Schonbeck U. & P. Libby: CD40 signaling and plaque instability. *Circ Res* 89, 1092-1103 (2001)

53. Inoue S, K. Egashira, W. Ni, S. Kitamoto, M. Usui, K. Otani, M. Ishibashi, K. Hiasa, K. Nishida & A. Takeshita: Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. *Circulation* 106, 2700-2706 (2002)

54. Lutgens E. & M.J. Daemen: CD40-CD40L interactions in atherosclerosis. *Trends Cardiovasc Med* 12, 27-32 (2002)

55. Rosenfeld M.E, P. Polinsky, R. Virmani, K. Kauser, G. Rubanyi & S.M. Schwartz: Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse. *Arterioscler Thromb Vasc Biol* 20, 2587-2592 (2000)

56. Johnson J.L. & C.L. Jackson: Atherosclerotic plaque rupture in the apolipoprotein E knockout mouse. *Atherosclerosis* 154, 399-406 (2001)

57. Nakata Y. & N. Maeda: Vulnerable atherosclerotic plaque morphology in apolipoprotein E-deficient mice unable to make ascorbic acid. *Circulation* 105, 1485-1490 (2002)

58. Murayama T, M. Yokode, H. Kataoka, T. Imabayashi, H. Yoshida, H. Sano, S. Nishikawa & T. Kita: Intraperitoneal administration of anti-c-fms monoclonal antibody prevents initial events of atherogenesis but does not reduce the size of advanced lesions in apolipoprotein E-deficient mice. *Circulation* 99, 1740-1746 (1999)

59. Rosenfeld M.E, K. Kauser, B. Martin-McNulty, P. Polinsky, S.M. Schwartz & G.M. Rubanyi: Estrogen inhibits the initiation of fatty streaks throughout the vasculature but does not inhibit intra-plaque hemorrhage and the progression of established lesions in apolipoprotein E deficient mice. *Atherosclerosis* 164, 251-259 (2002)

60. Rosenfeld M.E, K.G. Carson, J.L. Johnson, H. Williams, C.L. Jackson & S.M. Schwartz: Animal models of spontaneous plaque rupture: the holy grail of experimental atherosclerosis research. *Curr Atheroscler Rep* 4, 238-242 (2002)

61. Cullen P, R. Baetta, S. Bellostra, F. Bernini, G. Chinetti, A. Cignarella, A. von Eckardstein, A. Exley, M. Goddard, M. Hofker, E. Hurt-Camejo, E. Kanters, P. Kovanen, S. Lorkowski, W. McPheat, M. Pentikäinen, J. Rauterberg, A. Richie, B. Staels, B. Weitkamp & M. de Winther: Rupture of the atherosclerotic plaque: does a good animal model exist? *Arterioscler Thromb Vasc Biol* 23, 535-542 (2003)

62. Calara F, M. Silvestre, F. Casanada, N. Yuan, C. Napoli & W. Palinski: Spontaneous plaque rupture and secondary thrombosis in apolipoprotein E-deficient and LDL receptor-deficient mice. *J Pathol* 195, 257-263 (2001)

63. Zhou J, J. Møller, C.D. Danielsen, J. Bentzon, H.B. Ravn, R.C. Austin & E. Falk: Dietary supplementation with methionine and homocysteine promotes early atherosclerosis but not plaque rupture in apoE-deficient mice. *Arterioscler Thromb Vasc Biol* 21, 1470-1476 (2001)

64. Williams H, J.L. Johnson, K.G.S. Carson & C.L. Jackson: Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein E knockout mice. *Artherioscler Thromb Vasc Biol* 22, 788-792 (2002)

65. Jolley C.D, J.M. Dietschy & S.D. Turley: Genetic differences in cholesterol absorption in 129/Sv and C57BL/6 mice: effect on cholesterol responsiveness. *Am J Physiol* 39, G1117-G1124 (1999)

66. von der Thüsen J.H, B.J.M. van Vlijmen, R.C. Hoeben, M.M. Kockx, L.M. Havekes, T.J.C. van Berkel & E.A.L. Biessen: Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. *Circulation* 105, 2064-2070 (2002)

67. Ridker P.M, N. Rifai, L. Rose, J.E. Buring & N.R. Cook: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 347, 1557–1565 (2002)

68. Moreno P.R, A.M. Murcia, I.F. Palacios, M.N. Leon, V.H. Bernardi, V. Fuster & J.T. Fallon: Coronary composition and macrophage infiltration in atherectomy

specimens from patients with diabetes mellitus. *Circulation* 102, 2180-2184 (2000)

69. Bucala R, Z. Makita, T. Koschinsky, A. Cerami & H. Vlassara: Lipid advanced glycosylation: pathway for lipid oxidation *in vivo*. *Proc Natl Acad Sci USA* 90, 6434-6438 (1993)

70. Virella G, D. Atchley, S. Koskinen, D. Zheng & M.F. Lopes-Virella: Proatherogenic and proinflammatory properties of immune complexes prepared with purified human oxLDL antibodies and human oxLDL. *Clin Immunol* 105, 81-92 (2002)

71. Bucciarelli L.G, T. Wendt, W. Qu, Y. Lu, E. Lalla, L.L. Rong, M.T. Goova, B. Moser, T. Kislinger, D.C. Lee, Y. Kashyap, D.M. Stern & A.M. Schmidt: RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. *Circulation* 106, 2827-2835 (2002)

72. Sakaguchi T, S.F. Yan, S.D. Yan, D. Belov, L.L. Rong, M. Sousa, M. Andrassy, S.P. Marso, S. Duda, B. Arnold, B. Liliensiek, P.P. Nawroth, D.M. Stern, A.M. Schmidt & Y. Naka: Central role of RAGE-dependent neointimal expansion in arterial restenosis. *J Clin Invest* 111, 959-972 (2003)

73. Kim J.A, J.A. Berliner, R.D. Natarajan & J.L. Nadler: Evidence that glucose increases monocyte binding to human aortic endothelial cells. *Diabetes* 43, 1103-1107 (1994)

74. Schmidt A.M, O. Hori, J.X. Chen, J.F. Li, J. Crandall, J. Zhang, R. Cao, S.D. Yan, J. Brett & D. Stern: Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 96, 1395-1403 (1995)

75. Richardson M, S.J. Hadcock, M. DeReske & M.I. Cybulsky: Increased expression *in vivo* of VCAM-1 and E-selectin by the aortic endothelium of normolipemic and hyperlipemic diabetic rabbits. *Arterioscler Thromb* 14, 760-769 (1994)

76. Ribau J.C, S.J. Hadcock, K. Teoh, M. DeReske & M. Richardson: Endothelial adhesion molecule expression is enhanced in the aorta and internal mammary artery of diabetic patients. *J Surg Res* 85, 225-233 (1999)

77. Wong B.W, D. Wong & B.M. McManus: Characterization of fractalkine (CX3CL1) and CX3CR1 in human coronary arteries with native atherosclerosis, diabetes mellitus, and transplant vascular disease. *Cardiovasc Pathol* 11, 332-338 (2002)

78. Galis Z.S & J.J. Khatri: Matrix metalloproteinases in vascular remodeling and atherogenesis. The good, the bad, and the ugly. *Circ Res* 90, 251-262 (2002)

79. Galis Z, G. Sukhova & P. Libby: Microscopic localization of active proteases by *in situ* zymography: detection of matrix metalloproteinase activity in vascular tissue. *FASEB J* 9, 974-980 (1995)

80. Marx N, J. Froehlich, L. Siam, J. Ittner, G. Wierse, A. Schmidt, H. Scharnagl, V. Hombach & W. Koenig W: Antidiabetic PPAR□-activator rosiglitazone reduces MMP-9 serum levels in type 2 diabetic patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 23, 283-288 (2003)

81. Uemura S, H. Matsushita, W. Li, A.J. Glassford, T. Asagami, K.H. Lee, D.G. Harrison & P.S. Tsao: Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ Res* 88, 1291-1298 (2001)

82. Portik-Dobos V, M.P. Anstadt, J. Hutchinson, M. Bannan & A. Ergul: Evidence for a matrix metalloproteinase induction/activation system in arterial vasculature and decreased synthesis and activity in diabetes. *Diabetes* 51, 3063-3068 (2002)

83. Shi G.P, J.S. Munger, J.P. Meara, D.H. Rich & H.A. Chapman: Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease. *J Biol Chem* 267, 7258-7262 (1992)

84. Kafienah W, D. Bromme, D.J. Buttle, L.J. Croucher & A.P. Hollander: Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. *Biochem J* 331, 727-732 (1998)

85. Reddy V.Y, Q.Y. Zhang & S.J. Weiss: Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L, and S, by human monocyte-derived macrophages. *Proc Natl Acad Sci USA* 92, 3849-3853 (1995)

86. Sukhova G.K, G.P. Shi, D.I. Simon, H.A. Chapman & P. Libby: Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest* 102, 576-583 (1998)

87. Jormsjö S, D.M. Wuttge, A. Sirsjö, C. Whatling, A. Hamsten, S. Stemme & P. Eriksson: Differential expression of cysteine and aspartic proteases during progression of atherosclerosis in apolipoprotein E-deficient mice. *Am J Pathol* 161, 939-945 (2002)

88. Chen J, C.-H. Tung, U. Mahmood, V. Ntziachristos, R. Gyurko, M.C. Fishman, P.L. Huang & R. Weissleder: *In vivo* imaging of proteolytic activity in atherosclerosis. *Circulation* 105, 2766-2771 (2002)

89. Gacko M. & S. Glowinski: Cathepsin D and cathepsin L activities in aortic aneurysm wall and parietal thrombus. *Clin Chem Lab Med* 36, 449-452 (1998)

90. Shi G.P, G.K. Sukhova, A. Grubb, A. Ducharme, L.H. Rhode, R.T. Lee, P.M. Ridker, P. Libby & H.A. Chapman:

Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest* 104, 1191-1197 (1999)

91. Schaub F.J, D.K. Han, W.C. Liles, L.D. Adams, S.A. Coats, R.K. Ramachandran, R.A. Seifert, S.M. Schwartz & D.F. Bowen-Pope: Fas/FADD-mediated activation of a specific program of inflammatory gene expression in vascular smooth muscle cells. *Nature Med* 6, 790-796 (2000)

92. Fazio S, A.S. Major, L.L. Swift, L.A. Gleaves, M. Accad, M.F. Linton & R.V.Jr. Farese: Increased atherosclerosis in LDL receptor-null mice lacking ACAT1 in macrophages. *J Clin Invest* 107, 163-171 (2001)

93. Nhan T.Q, W.C. Liles, A. Chait, J.T. Fallon & S.M. Schwartz: The p17 cleaved form of caspase-3 in present within viable macrophages *in vitro* and in atherosclerotic plaque. *Arterioscler Thromb Vasc Biol* May 22 [epub ahead of print] (2003)

94. Kubo N, L.K. Curtiss, S. Yamada, H. Kanno, I. Sakurabayashi, K. Itoh & W.A. Boisvert: Role of macrophage apoptosis in atherogenesis. *Arterioscler Thromb Vasc Biol* 22, p317, Abstr. (2002)

95. Kockx M.M. & A.G. Herman: Apoptosis in atherosclerosis: beneficial or detrimental? *Cardiovasc. Res* 45, 736-746 (2000)

96. Rekhter M.D, G.W. Hicks, D.W. Brammer, H. Hallak, E. Kindt, J. Chen, W.S. Rosebury, M.K. Anderson, P.J. Kuipers & M.J. Ryan: Hypercholesterolemia causes mechanical weakening of rabbit atheroma: local collagen loss as a prerequisite of plaque rupture. *Circ Res* 86, 101-108 (2000)

97. Geng Y.J. & P. Libby: Evidence of apoptosis in advanced human atheroma. Colocalization with interleukin-beta converting enzyme. *Am J Pathol* 147, 251-266 (1995)

98. McCarthy N.J. & M.R. Bennett: The regulation of vascular smooth muscle apoptosis. *Cardiovasc Res* 45, 747-755 (2000)

99. Kockx M.M, G.R. De Meyer, N. Buyssens, M.W. Knaapen, H. Bult & A.G. Herman: Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal. *Circ Res* 83, 378-387 (1998)

100. Rong J.X, J. Li, E.D. Reis, R.P. Choudhury, H.M. Dansky, V.I. Elmalem, J.T. Fallon, J.L. Breslow & E.A. Fisher: Elevating high-density lipoprotein cholesterol in apolipoprotein E-deficient mice remodels advanced atherosclerotic lesions by decreasing macrophage and increasing smooth muscle cell content. *Circulation* 104, 2447-2452 (2001)

101. Aikawa M, E. Rabkin, Y. Okada, S.J. Voglic, S.K. Clinton, C.E. Brinckerhoff, G.K,. Sukhova & P. Libby: Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content in rabbit atheroma: a potential mechanism of lesion stabilization. *Circulation* 97, 2433-2444 (1998)

102. Bennett M.R, G.I. Evan & S.M. Schwartz: Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. *J Clin Invest* 95, 2266-2274 (1995)

103. Bennett M.R: Apoptosis in the cardiovascular system. *Heart* 87, 480-487 (2002)

104. Chu Y, F.M. Faraci, H. Ooboshi & D.D. Heistad: Increase in TUNEL positive cells in aorta from diabetic rats. *Endothelium* 5, 241-50 (1997)

105. Hall J.L, C. Matter, M.J. Pollman, H.Z. Bai, Y. Inishi & G.H. Gibbons: Hyperglycemia inhibits vascular smooth muscle cell apoptosis through a protein kinase C-dependent pathway. *Circulation* 98, 574–580 (2000)

106. Peiro C, N. Lafuente, N. Matesanz, E. Cercas, J.L. Llergo, S. Vallejo, L. Rodriguez-Manas & C.F. Sanchez-Ferrer: High glucose induces cell death of cultured human aortic smooth muscle cells through the formation of hydrogen peroxide. *Br J Pharmacol* 133, 967-974 (2001)

107. Hall J.L, J.C. Chatham, H. Eldar-Finkelman & G.H. Gibbons: Upregulation of glucose metabolism during intimal lesion formation is coupled to the inhibition of vascular smooth muscle cell apoptosis. Role of GSK3beta. *Diabetes* 50, 1171-1179 (2001)

108. Sakuma H, M. Yamamoto, M. Okumura, T. Kojima, T. Maruyama & K. Yasuda: High glucose inhibits apoptosis in human coronary artery smooth muscle cells by increasing bcl-xL and bfl-1/A1. *Am J Physiol Cell Physiol* 283, C422-C428 (2002)

109. Gerrity R.G, R. Natarajan, J.L. Nadler & T. Kimsey: Diabetes-induced accelerated atherosclerosis in swine. *Diabetes* 50, 1654-1665 (2001)

110. Oldstone M.B, M. Nerenberg, P. Southern, J. Price & H. Lewicki: Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* 65, 319-331 (1991)

111. von Herrath M.G, J. Dockter & M.B. Oldstone: How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. *Immunity* 1, 231-242 (1994)

112. Breddin H, H. Krzywanek, P. Althoff, K. Schoffling & K. Ubeila: PARD: Platelet aggregation as a risk factor in diabetes: results of a prospective study. *Horm Metab Res* 15, 63-68 (1985)

113. Colwell J & M. Lopes-Virella: A review of the development of large vessel disease in diabetes mellitus. *Am J Med* 85, 113-119 (1988)

114. Ziboh V, H. Maruta, J. Lord, W. Cagle & W. Lucky: Increased biosynthesis of thromboxane A2 by diabetic platelets. *Eur J Clin Invest* 9, 223-228 (1979)

115. Butkus A, A. Skrinska & O. Schumacher: Thromboxane production and platelet aggregation in diabetic subjects with clinical complications. *Thromb Res* 19, 211-223 (1980)

116. Lagarde M, M. Burtin, P. Berciaud, M. Blanc, B. Velardo & M. Dechavanne: Increase of platelet thromboxane A2 formation and its plasmatic half-life in diabetes mellitus. *Thromb Res* 19, 823-830 (1980)

117. Davi G, I. Catalano, M. Averna, A. Notobartolo, A. Strano & G. Ciabottoni: Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N Engl J Med* 322, 1769-1774 (1990)

118. Ostermann H. & J. van de Loo: Factors of the hemostatic system in diabetic patients. *Haemostasis* 16, 386-416 (1986)

119. Sagel J, J. Colwell, L. Crook & M. Laimans: Increased platelet aggregation in early diabetes mellitus. *Ann Intern Med* 82, 733-738 (1975)

120. Badawi H, M. El-Sawy, M. Mikhail, A. Nomier & S. Tewfik: Platelets, coagulation in diabetic and nondiabetic patients with quiescent coronary heart disease. *Angiology* 21, 511-519 (1970)

121. Rosove M, F. Harrison & M. Harwig: Plasma B-thromboglobulin, platelet factor 4, fibrinopeptide A, and other hemostatic functions during improved short-term glycemic control in diabetes mellitus. *Diabetes Care* 7, 174-179 (1984)

122. Small M, G. Lowe, A. MacCuish & C. Forbes: Thrombin and plasmin activity in diabetes mellitus and their association with glycemic control. *Q J Med* 248, 1025-1031 (1987)

123. Sobel B.E: Fibrinolysis and diabetes. *Front Biosci* 8, d1085-1092 (2003)

124. Herlihy O.M, B.A. Barrow, P.J. Grant & J.C. Levy: Hyperglycaemic siblings of Type II (non-insulin-dependent) diabetic patients have increased PAI-1, central obesity and insulin resistance compared with their paired normoglycaemic sibling. *Diabetologia* 45, 635-641 (2002)

125. Lapolla A, F. Piarulli, G. Sartore, C. Rossetti, L. Martano, P. Carraro, M. De Paoli & D. Fedele: Peripheral artery disease in type 2 diabetes: the role of fibrinolysis. *Thromb Haemost* 89, 91-96 (2003)

126. Eren M, C.A. Painter, J.B. Atkinson, P.J. Declerck & D.E. Vaughan: Age-dependent spontaneous coronary arterial thrombosis in transgenic mice that express a stable form of human plasminogen activator inhibitor-1. *Circulation* 106, 491-496 (2002)

127. Yuan C, S.X. Zhang, N.L. Polissar, D. Echelard, G. Ortiz, J.W. Davis, E. Ellington, M.S. Ferguson & T.S. Hatsukami: Identification of fibrous cap rupture with magnetic resonance imaging is highly associated with recent transient ischemic attack or stroke. *Circulation* 105, 181-185 (2002)

128. Yuan C, W.S. Kerwin, M.S. Ferguson, N. Polissar, S. Zhang, J. Cai & T.S. Hatsukami: Contrast-enhanced high resolution MRI for atherosclerotic carotid artery tissue characterization. *J Magn Reson Imaging* 15, 62-67 (2002)

129. Fayad Z.A & V. Fuster: The human high-risk plaque and its detection by magnetic resonance imaging. *Am J Cardiol* 88, 42E-45E (2001)

130. Hatsukami T.S, R. Ross, N.L. Polissar & C. Yuan: Visualization of fibrous cap thickness and rupture in human atherosclerotic carotid plaque *in vivo* with high-resolution magnetic resonance imaging. *Circulation* 102, 959-964 (2000)

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; ACE, angiotensin-converting-enzyme; AGE, advanced glycation endproducts; apoE, apolipoprotein E; CRP, C-reactive protein; CSF, colony stimulating factor; DCCT, the Diabetes Control and Complications Trial; FADD, Fas-associating death domain protein; GP, lymphocytic choriomeningitis virus glycoprotein; HbA1c; glycated hemoglobin A1c; HDL, high-density lipoprotein; IL-1beta, interleukin 1beta; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LCMV, lymphocytic choriomeningitis virus; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; PAI-1, plasminogen activator inhibitor-1; PDAY study, the Pathobiological Determinants of Atherosclerosis in Youth study; PDGF, platelet-derived growth factor; RAGE, receptor for advanced glycation endproducts; RIP, rat insulin promoter; SMC, smooth muscle cell; TIMP, tissue inhibitor of metalloproteinases; TNFalpha, tumor necrosis factor alpha; TUNEL, terminal transferase-mediated dUTP nick end-labeling; UKPDS, United Kingdom Prospective Diabetes Study; VCAM-1, vascular cell adhesion molecule-1; VLDL, very low-density lipoprotein.

Key Words: Atherosclerosis, Inflammation, Matrix metalloproteinases, Mouse models, Thrombosis, Review

Send correspondence to: Dr Karin E. Bornfeldt, Department of Pathology, Box 357470, University of Washington School of Medicine, Seattle, WA 98195-7470, Tel: 206-543-1681, Fax: 206-543-3644, email: bornf@u.washington.edu