Point of View

# The Response-to-Retention Hypothesis of Early Atherogenesis

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Many processes have been implicated in early atherogenesis. These include endothelial denudation, injury, or activation, including shear stress–related events; local adherence of platelets; lipoprotein oxidation; lipoprotein aggregation; macrophage chemotaxis and foam cell formation; and smooth muscle cell alterations. Which process, if any, could be regarded as the key event in early atherogenesis, ie, absolutely required, yet also sufficient as the sole pathological stimulus in an otherwise normal artery to provoke a cascade of events leading to lesion formation? The work of many investigators, which we summarize here, strongly supports subendothelial retention of atherogenic lipoproteins as the central pathogenic process in atherogenesis (for prior reviews, see References 1 through 6). Our thesis is that other contributory processes are either not individually necessary or are not sufficient. Most often, they are merely normal, expected responses of otherwise-healthy tissue to the presence of retained lipoproteins.

## Competing Hypotheses

It is instructive to catalog other processes that have been argued to be central to the initiation of atherogenesis. The first is endothelial denudation,[7-9] injury,[10] or activation,[11,12] as outlined in the "response-to-injury" hypothesis of Ross, Glomset, and coworkers. Although this important hypothesis has stimulated much of the work that we cite here, there is no definitive evidence in vivo that endothelial injury is either necessary or sufficient for lesion formation.

The response-to-injury hypothesis originally presupposed endothelial desquamation as the key event in atherogenesis.[7-9] It is now clear, however, that developing atheromata are covered by an intact endothelial layer throughout most stages of lesion progression: lipoprotein retention, fatty streak formation, and formation of advanced lesions.[5,6,12-15] In humans, only the most complicated, ulcerated lesions lose their endothelial layer. Furthermore, in some experimental models, an intact endothelium is required for lesion initiation and development, which do not occur in adjacent areas of denudation.[16-18] Gross endothelial denudation, though presumably important in restenosis after balloon injury[19] and in very advanced complicated plaques, does not appear to be central to early atherogenesis.[5]

A refinement of the response-to-injury hypothesis states that endothelial injuries that are insufficient to cause gross denudation but severe enough to cause functional modifications are key to atherogenesis.[10-12,20] A major hypothesized change in endothelial function was increased permeability,[21,22] particularly to atherogenic lipoproteins.[23-27] This idea is related to the lipid infiltration hypothesis,[5] which originated with Anichkov and Khalatov[28] (reviewed in References 29 and 30). Alterations in permeability or even microscopic losses of endothelial cells in excess of those due to normal cell turnover are not mechanistically required for atherogenesis, however, because normal, healthy endothelium transports[31,32] or "leaks"[26,33] many molecules, including lipoproteins (reviewed in Reference 27). In fact, the rate of LDL entry into the normal, healthy arterial wall vastly exceeds the LDL accumulation rate.[34] Most important, seminal studies by Schwenke and Carew[35,36] showed in vivo that the early prelesional accumulation of atherogenic lipoproteins within the arterial wall is focally concentrated in sites that are known to be prone to the later development of atheromata, but that the rates of lipoprotein entry into prelesional susceptible versus resistant sites were not different (cf Reference 2). These studies indicate that retention, not enhanced endothelial permeability to lipoprotein influx, is the key pathological event in this experimental model. Subsequent studies in several other animal models have demonstrated either increased[23-26] or decreased[37] rates of lipoprotein entry into atherosclerosis-susceptible sites, suggesting a nonessential role for alterations in endothelial permeability. All studies agree, however, that prelesional susceptible arterial sites show enhanced retention of apoB-rich, atherogenic lipoproteins.[35,36,38-41] We conclude that alterations in endothelial permeability, though apparently not essential to lesion development, may play a contributory role, eg, in smoking,[42] dyslipidemias[27,37] (cf Reference 36), and possibly hypertension[27] (cf Reference 43), but only if some of the infiltrated material is retained.[35]

Several other functional modifications have been documented in the endothelial layer in vivo during atherogenesis, but these occur comparatively late. For example, in rabbits, cell adhesion molecules,[44] the earliest known being vascular cell adhesion molecule–1 (VCAM-1), are expressed by endothelial cells that overlie lesions, but only after more than 4 days of severe hypercholesterol-

Received December 5, 1994; accepted February 21, 1995.

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emia and resultant foam cell formation.[45] In contrast, lipoprotein retention and aggregation are detectable within minutes to hours after the onset of hypercholesterolemia.[31,36,41,46] Furthermore, atherogenic lipoproteins and their components have been shown to regulate endothelial expression of cell-adhesion molecules.[12,47-49] The most straightforward conclusion is that the earliest known endothelial changes during atherogenesis in vivo, such as VCAM-1 expression, cannot be a cause, and are likely to be a consequence, of the initial retention of lipoproteins within the arterial wall (see "Future Directions").

The effect of turbulent blood flow on the arterial wall deserves special comment, particularly because it can be such an early influence. Arterial segments that are subject to turbulent blood flow, such as those at branch points or during hypertension, show a predisposition to lesion development, though the precise relationship in vivo may be complicated.[50] Because of the response-to-injury hypothesis, the connection between blood flow and atherogenesis has led to many studies on the effects of shear stress on the endothelium in cell culture experiments. Many alterations have been reported,[12,18,51] such as intracellular calcium mobilization, ion channel activation, cytoskeletal changes, altered cellular alignment, cell surface streamlining,[52] increased endothelial cell division,[53] stimulation of specific transcription factors,[54] and production of potentially atherogenic molecules, such as vasoactive,[55] adhesive,[56] and growth[12,20,57] factors. Somewhat different results are obtained in vitro when shear is low instead of high, constant instead of pulsatile, laminar instead of turbulent, or spatially uniform instead of graded,[12,51,53] but the overall findings in vitro strongly support a contributory role for shear stress–induced alterations of the endothelium during atherogenesis.

In vivo, however, it is clear that sheer stress–induced endothelial alterations are neither necessary nor sufficient for atherogenesis. In vivo lesion development at sites of turbulent flow shows an absolute requirement for high plasma concentrations of atherogenic lipoproteins relative to those that occur naturally in nonhuman, nonatherosclerotic mammals: the plasma concentration of LDL cholesterol must exceed 2 mmol/L (80 mg/dL) for atherogenesis, even at sites of high shear stress.[58,59] Furthermore, at sufficiently high plasma lipoprotein concentrations, lesions develop even at sites of low shear stress, such as at non–branch points[6,60] or within the pulmonary arteries.[60,61] Although stress-induced endothelial changes can play a contributory role in atherogenesis, the most directly relevant functional changes that have been documented at prelesional sites that are susceptible to atherogenesis, including those that are subject to turbulent blood flow, are altered proteoglycan structure[62-66] and increased lipoprotein retention[36,39,46,67-69] (see above).

We therefore propose that the atherogenic effects of sheer stress in vivo are entirely dependent on lipoprotein retention within the arterial wall and are limited to increased local vulnerability to lipoprotein retention and the consequences thereof. Specifically, we hypothesize that the role of shear stress in early atherogenesis is mediated primarily through the stimulation of intramural synthesis of molecules, such as proteoglycans, that promote lipoprotein retention (see References 63, 64, 66, 70, and 71). Later, once vessel segments have accumulated retained lipoproteins, the threshold for injury and activation from continued shear stress may be lowered. Many stimuli can activate endothelial cells, and synergy is likely between shear stress and, for example, oxidative breakdown products of retained lipoproteins. The same general lines of reasoning can be used to argue against a central role for other potential activators of the endothelium, such as viruses[72] or homocysteine,[9,12,20,73] which are likewise neither necessary nor sufficient for lesion development in vivo. Note that these hypotheses about the central role of retained lipoproteins are testable (see "Future Directions").

The second process that has been proposed to be central to atherogenesis is lipoprotein oxidation.[74-76] Current evidence indicates, however, that pathophysiologically important oxidation can occur only after the retention of lipoproteins within the sequestered microenvironment of the arterial wall. Lipoprotein oxidation by cells or transition metals in vitro is blocked by small concentrations of plasma or plasma proteins,[77] such as albumin[78-83] (cf Reference 84), and any oxidized lipoproteins that might appear in the plasma in vivo would be rapidly removed by the liver,[85,86] rather than be deposited into developing lesions within the arterial wall.[87] In fact, oxidation may be regarded as a normal, expected consequence of lipoprotein trapping: studies in vitro indicate that once lipoproteins are sequestered from the protective elements of plasma, nearby healthy arterial cells will cause oxidation,[75] apparently through their efficient generation of thiols.[88,89] In vivo, myeloperoxidases may be involved.[90] Note that adherence of LDL to arterial proteoglycans increases LDL's susceptibility to oxidation in vitro[71,91] (see below), but that prior oxidation of LDL does not enhance its retention in arteries.[87] Consistent with these results, apoB is retained in the human intima before it is detectably oxidized.[92] The most straightforward conclusion is that oxidation is a normal, expected consequence of intramural sequestration of sufficient quantities of atherogenic lipoproteins within an otherwise healthy artery.

The importance of lipoprotein oxidation in lesion development is supported by discoveries of many biological consequences in vitro that are consistent with atherogenesis,[48,74,75,93] by demonstrations of antiatherogenic effects in experimental animals of compounds with antioxidant actions,[94-96] and by the findings in humans of oxidized epitopes within lesions[92,97] (see Reference 98) and of anti–oxidized LDL antibodies within lesions[99] and in plasma.[100] Nevertheless, there are also reports that show the ineffectiveness of antioxidants on atherogenesis. For example, recent reports of humans who consumed antioxidant vitamins[101,102] or who were given probucol[103,104] and of animals that were given a potent antioxidant analogue of probucol that does not affect plasma lipoprotein concentrations[105,106] (see Reference 107) failed to find beneficial effects of these treatments on disease. Note that vitamin E and probucol, which appear to be antiatherogenic in humans[102] and animals,[94,95] respectively, have many actions besides inhibition of lipoprotein oxidation.[102,104,105,108,109] Because even minimal oxidation of LDL, which has been described in human lesions[110] (see also Reference 111), produces many potentially harmful biological effects,[93] lipoprotein oxidation is unlikely to be a rate-limiting step in atherogenesis. Thus, nearly total inhibition of oxidation of

retained lipoproteins may be required before there would be any substantial effect on lesion development in vivo (cf Reference 105).

## Evidence to Support Retention as the Key Event

Following rapid induction of hypercholesterolemia in rabbits due to injection of LDL, the earliest detectable change in the arterial wall is the intramural retention of LDL and of microaggregates of LDL, a change that occurs within 2 hours.[46] Perfusion of arterial segments in situ has shown substantial accumulation of LDL within 5 minutes.[31] Early arterial retention of injected LDL in vivo is focal, in sites that are known to be susceptible to the subsequent development of atheromata.[35,36] LDL retention in these sites is not the result of increased flux into the arterial wall but from reduced lipoprotein egress.[36] These rapidly apparent differences in lipoprotein retention between prelesional susceptible versus resistant sites suggest a preexisting metabolic difference that, under the proper conditions of hypercholesterolemia, leads to differences in lipoprotein retention. This conclusion is supported by the observation that atherosclerosis cannot develop when plasma $\beta$-lipoprotein concentrations are truly low,[58,59,112] even in the presence of other major risk factors.[113]

Several lines of evidence indicate that intramural retention of atherogenic lipoproteins involves extracellular matrix, chiefly proteoglycans[1,71] and perhaps other structural elements,[114-119] and lipolytic enzymes, chiefly lipoprotein lipase (LpL)[120-127] and sphingomyelinase (SMase).[127-130] First, all of these molecules are present to varying degrees within the normal arterial wall.[70,71,120,128,131-133] Thus, they are available to participate in the earliest stages of atherogenesis. Second, retained apoB in extremely early[46,69] as well as advanced[64,71] lesions is closely associated with arterial proteoglycans. Purified arterial proteoglycans, particularly those from lesion-prone sites,[66,134] bind LDL in vitro, particularly LDL from patients with coronary artery disease.[135,136] This interaction involves several well-defined, positively charged segments of apoB.[71,137] Third, LpL enhances adherence of LDL in vitro to the matrix that is derived from normal endothelial[125,138] and smooth muscle[127] cells and to normal cell-surface proteoglycans.[122-127,139] This adherence is independent of LpL enzymatic activity[122,123,125,127] (cf References 120 and 121) and appears to occur in vessels enriched in situ with LpL.[140] Fourth, a linkage between peritoneal macrophage production of LpL and susceptibility to atherosclerosis has been documented in recombinant inbred mice.[141] Results in humans indicate a linkage between LpL polymorphisms and coronary artery disease, though without a linkage between LpL polymorphisms and specific plasma lipoprotein patterns, thus suggesting that the effects on lesion development are independent of plasma changes.[142] A genetic absence of LpL in humans has long been known to cause hyperlipidemia without increased atherosclerosis,[121] presumably because of poor generation of small, cholesteryl ester–rich particles[143] that are able to enter the arterial wall[27] and loss of LpL-facilitated binding to arterial proteoglycans.[122-127] More recent work in humans indicates that the single most important determinant of lesion development in homozygous familial hypercholesterolemia is the postheparin plasma concentration of LpL mass, not enzymatic activity; this is consistent with a structural effect.[144] Fifth, SMase causes the formation of LDL microaggregates[129] that morphologically resemble the intramural particles seen 2 hours after rapid induction of hypercholesterolemia,[46] and LpL and SMase synergistically interact to cause massive retention and aggregation of LDL and Lp(a) in vitro to arterial cell proteoglycans and matrix.[127] The arterial wall SMase[128] was recently shown to act on the LDL retained in aortic strips ex vivo.[130] At later stages, once atherogenesis has begun, the content of specific proteoglycans,[64-66,71,145] LpL,[120,131-133,146-148] and SMase[128] increases in lesional areas, thereby apparently accelerating the disease.

The factors responsible for focal retention of lipoproteins and subsequent lesion development, however, are not clear. Because prelesional differences in lipoprotein retention between susceptible versus resistant arterial sites are so rapidly apparent after induction of hypercholesterolemia,[35] there are likely to be preexisting local differences in apoB-retentive molecules. Proteoglycan variations alone may not explain the focal development of early lesions, because potentially atherogenic proteoglycans are abundant and ubiquitous throughout the arterial tree,[70] though focal alterations in proteoglycans have been documented in prelesional[63-66] and lesional[145,149] sites. The enzymes LpL and SMase show enhanced expression in established human[133,146,148] and animal[133] lesions, and arterial contents of LpL were shown two decades ago to correlate strongly with the arterial accumulation of cholesteryl ester during atherogenesis in rabbits ($r=0.9$).[121] Nevertheless, the status of proteoglycans, LpL, SMase, and possibly other apoB-retentive molecules in prelesional susceptible versus prelesional resistant sites remains an important area for study (see "Future Directions").

## Consequences of Retention

Following its retention by proteoglycans, LDL has been shown in vitro to undergo several modifications with important biological consequences. Proteoglycan-bound LDL in vitro forms aggregates[71] and vesicular structures[127] that resemble material seen in vivo.[46,67,68,150,151] LDL-proteoglycan complexes show increased susceptibility to oxidation under typical serum-free, albumin-free pro-oxidative experimental conditions.[91] Minimally oxidized LDL induces endothelial and smooth muscle cells to express monocyte chemotactic activity,[152] and more extensively oxidized LDL is directly chemoattractive to monocytes,[153,154] smooth muscle cells,[155] and T lymphocytes,[154] largely because of its lysophosphatidylcholine content.[154] Retained LDL would also be subject to arterial wall SMases,[127-130] which generate choline phosphate and ceramides. Ceramides are well documented to have many biological effects, such as induction of NF-$\kappa$B and stimulation of apoptosis or mitogenesis.[156,157]

LDL that has been aggregated or otherwise modified by arterial proteoglycans under a variety of conditions in vitro is avidly taken up by normal cultured macrophages and smooth muscle cells,[134] leading to foam cell formation.[136,151,158] This avid uptake may involve several different receptors,[122-126,134,139,158,159] of which only the LDL receptors are known to be nonessential, in that their genetic absence does not impede arterial lipoprotein

accumulation[160] or atherogenesis[60,161] in vivo, in contrast to the situation with LpL deficiency (see above). The conversion of macrophages to foam cells stimulates the release of more LpL[133,146-148,162,163] and other potentially atherogenic factors[164-166] and has been shown to alter proteoglycan metabolism.[167] Retained, altered lipoproteins[155,168,169] and nearby macrophages[170,171] can stimulate chemotaxis and transformation of smooth muscle cells from the contractile to the proliferative state, which in smooth muscle cells causes increased synthesis of proteoglycans,[64,172] including LDL-binding proteoglycans,[173] and possibly LpL[132,133] (cf Reference 146). Thus, retained lipoproteins can directly or indirectly provoke all known features of early lesions and, by stimulating local synthesis of proteoglycans and LpL, can accelerate further lipoprotein retention and aggregation.

Lesion development may be altered by local cellular expression of apoE within the arterial wall. As macrophages become foam cells in situ, they appear to increase their synthesis of apoE,[148,174] a molecule that has been shown in vitro to release lipoproteins from the extracellular matrix[175] (see Reference 137). The ultimate fate of these released lipoproteins, however, is unclear. They could leave the lesion, or they could be taken up by arterial cells, particularly through apoE-mediated binding.

The atherogenic nature of Lp(a) may originate from its extensive propensity for intramural retention.[41,136,176-178] Despite plasma concentrations of Lp(a) that are far lower than those of other apoB-rich lipoproteins, Lp(a) may account for most of the apoB in human lesions.[179,180] In vitro, Lp(a) binds to arterial proteoglycans with greater affinity[176] and capacity[176,181] than does LDL. Avid cellular uptake of Lp(a) by at least four processes may then occur: through scavenger receptors after oxidation[86]; through the heparan sulfate–proteoglycan pathway in the presence of intramural LpL[122,123]; by phagocytosis following aggregation and cross-linking by LpL, SMase, and proteoglycans[127]; and by a specific Lp(a)/apo(a) receptor on cholesterol-enriched macrophages.[182,183] After Lp(a) retention, many other biological effects occur, including enhanced LDL retention,[184] stimulation of smooth muscle cell proliferation,[185] and, possibly, local inhibition of lysis of microthrombi[186-189] (cf References 190 through 192).

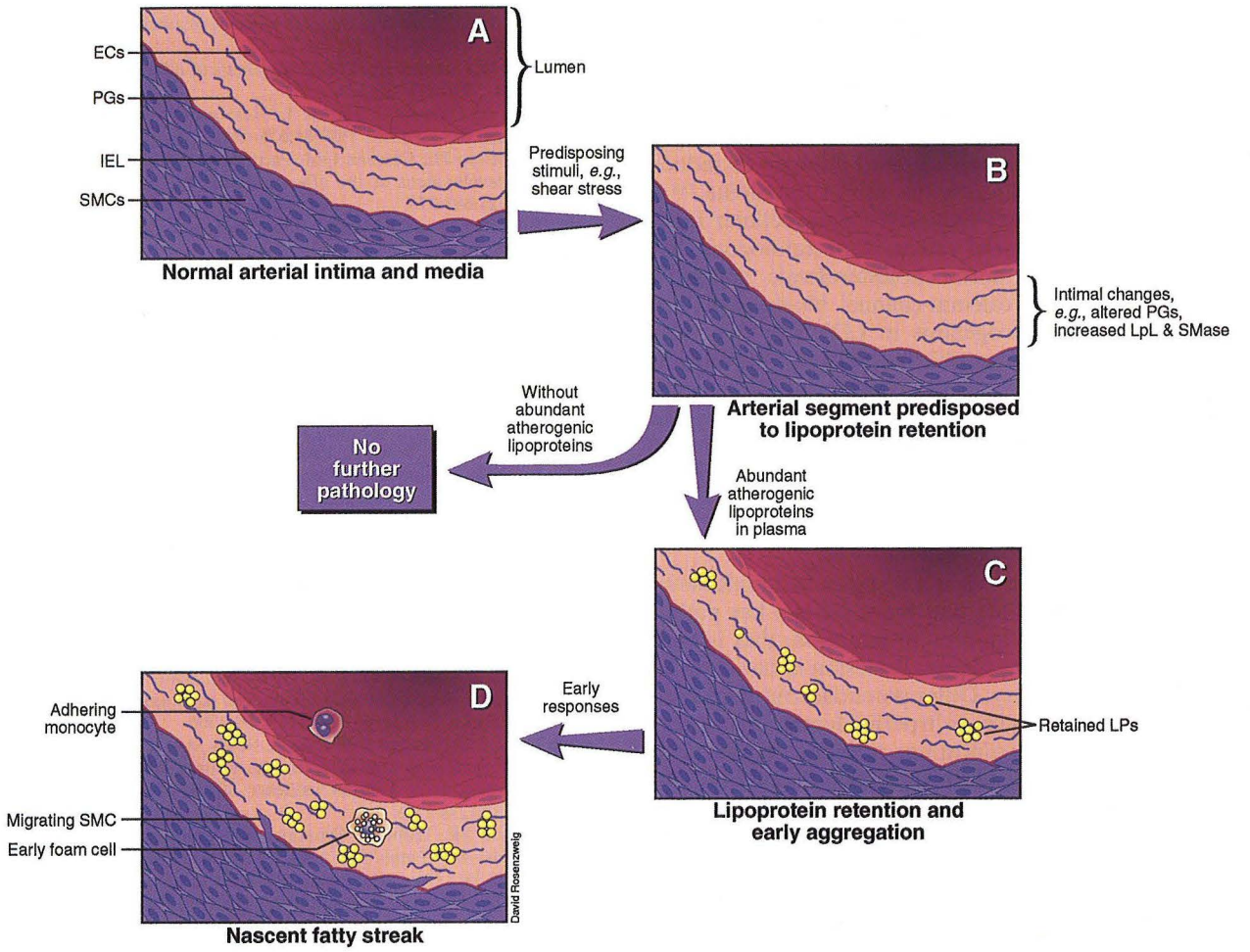
## Future Directions

A major mystery in atherogenesis is the well-known variation in lesion progression among individuals with similar plasma lipid profiles (see Reference 59) and among different arterial sites within the same individual.[6] All known risk factors account for merely 50% of coronary events and well under 50% of interindividual variability in the actual extent of coronary lesions.[193] The remaining risk is at least partially attributable to poorly characterized properties of the vessel wall. The response-to-retention hypothesis predicts that these vessel wall factors include molecules involved in lipoprotein retention, which, at this state of our knowledge, means proteoglycans, LpL, SMase, apoE, apoB, and apo(a).

Tools now exist or can be developed to assess the roles of proteoglycans and arterial wall enzymes in very early atherogenesis. Many proteoglycan core proteins of endothelial[194-196] and smooth muscle[194,195,197-202] cells have been cloned and sequenced, which would allow linkage studies in animals and humans and direct genetic manipulation, particularly at lesion-susceptible branch points. Enzymes that are involved in proteoglycan side-chain assembly are still being characterized,[203-206] but we suggest that polymorphisms might play a role in atherosclerosis susceptibility (see References 207 and 208). To establish a causal role for LpL in atherogenesis in vivo, direct stimulation and especially suppression of intramural arterial LpL must be done, followed by an examination of lesion progression (see Reference 140). Because several functional domains within LpL have already been characterized,[209] specific constructs can be engineered to separately examine in vivo the atherogenicity of its enzymatic[121] versus structural[122,123,125,127] actions. Two decades ago, lesions were shown to be enriched with SMase, but detailed enzymatic or molecular characterization of this lipase activity is still lacking, thus preventing linkage studies or genetic manipulation. The mechanism of SMase-induced aggregation in vitro has been shown to depend on the generation of ceramide.[130] Thus, to implicate SMase in atherogenesis, lesional lipoproteins will have to be shown to be enriched in ceramide.

Investigation into the local roles of apoproteins and other molecules in very early atherogenesis can also be performed. Transgenic mice that overexpress apoE in the arterial wall, among other sites, have shown reduced atherosclerosis in one preliminary report,[210] consistent with the hypothesis of a local protective role for this molecule in vivo.[175,211] Transgenic mice that overexpress apoA-I show reduced lesion development,[212-214] perhaps because of accelerated reverse cholesterol transport, but possibly because of the local inhibitory effects that apoA-I particles exert on aggregation[215,216] and oxidation[77,80,84,215,217] of atherogenic lipoproteins. It will be interesting to develop animal models in which overexpression of apoE or apoA-I is cleanly confined to the arterial wall. The physical characteristics of LDL, including its size,[218,219] apoB conformation,[220] or sialic acid content,[221] may affect binding affinity to arterial proteoglycans[135,219,221] and subsequent oxidation.[220,222] Apo(a) polymorphisms[177] should also be examined.

Tools exist as well to investigate the proposed sequence of events in early atherogenesis, subsequent to lipoprotein retention. For example, on the basis of the apparent sequence of events in vivo, we predict that massive retention of LDL or Lp(a) by smooth muscle cells or matrix[127] will stimulate nearby endothelial and possibly smooth muscle cells[127,152,223] to express cell adhesion molecules, chemoattractants, and growth factors (see "Competing Hypotheses"). A likely mechanism would be oxidation of the retained lipoproteins, followed by release of biologically active components, such as lysophosphatidylcholine, which is known to stimulate the expression of VCAM-1, platelet-derived growth factor, and other molecules by otherwise-normal endothelium.[224] The prediction can be tested by coculture in vitro,[84] with specific emphasis on the search for lipoprotein retention as an initial event. In addition, we predict that one key atherogenic effect of turbulent blood flow on prelesional sites in vivo is the locally enhanced expression of apoB-retaining molecules, particularly by vascular smooth muscle cells. A search for these molecules in susceptible versus resistant prelesional arterial sites could be undertaken, the molecules genetically manipulated if already cloned, and the effects on atherogenesis examined. Possible mechanisms for altered ex-




Schematic of the response-to-retention model of early atherogenesis. Mild to moderate hyperlipidemia causes lesion development only in specific sites within the arterial tree, implying the existence of predisposing stimuli, such as sheer stress, that make these sites particularly lesion-prone by stimulating local synthesis of apoB-retentive molecules (B). Predisposing stimuli in the absence of abundant atherogenic lipoproteins (ie, <2 mmol LDL cholesterol/L) are insufficient to cause atherogenesis. Predisposing stimuli in the presence of abundant atherogenic lipoproteins result in lipoprotein retention (C). Evidence suggests that aggregation promptly follows or may be part of the retentive process. Once significant retention has occurred, a cascade of early responses, including lipoprotein oxidation and cellular chemotaxis, leads to lesion development (D). ECs indicates endothelial cells; PGs, proteoglycans; IEL, internal elastic lamina; SMCs, smooth muscle cells; LpL, lipoprotein lipase; SMase, sphingomyelinase; and LPs, lipoproteins.

pression of these molecules would include direct effects of hemodynamic forces acting through sheer stress response–elements in underlying smooth muscle cells[18] or through endothelium-dependent effects,[63] including direct electrical communication between the endothelium and smooth muscle[18,225] or humoral signals.[63] For example, transforming growth factor–$\beta$ is expressed by the endothelium under control of a shear stress–responsive element[57] and is known to stimulate synthesis of chondroitin sulfate proteoglycans by smooth muscle cells.[226,227]

Finally, the search for additional molecules or mechanisms that may be important in vivo to lipoprotein retention and responses to retention should continue. For example, collagen,[114] fibrin,[115,116] fibronectin,[117-119] and matrix-bound phospholipase $A_2$[228,229] have been implicated in several studies. Also, the LDL receptor–related protein, which binds LpL[230] and apoE[231] on ligand blots in vitro, has recently been reported to be present in normal and lesional arteries.[232,233] Of particular interest is how an arterial segment might remain healthy after the entry of lipoproteins (see Reference 92). For example, there is substantial and well-documented evidence for the egress of atherogenic lipoproteins from the normal arterial wall,[27,36] which has generally been assumed to be passive, though it may not be. Other processes that could blunt or enhance oxidative and inflammatory responses to retained lipoproteins show genetic variability in mice[234] and merit further study in humans.

Arterial retention of atherogenic lipoproteins is a logical target for therapeutic intervention. So far, three strategies specifically directed against lipoprotein retention have been proposed in the literature. The first strategy is the local use of molecules that interfere with adherence of apoB or apo(a)-apoB to arterial proteoglycans. As noted before, apoE is a promising candidate, in vitro[137,175] and possibly in mice.[210] Boosting local expression of apoE in human arterial segments, however, would be difficult at present and may require gene therapy, an approach still in its infancy. Other potential candidates include apoB fragments[137] or other proteo-

glycan-binding peptides.[235-237] The second strategy proposed in the literature is inhibition of intra-arterial SMase activity.[129] The effects of SMase, unlike the effect of LpL, involve its enzymatic action.[130] Specific enzymatic inhibitors could test its role in vivo and might provide therapeutic benefit. A third strategy to reduce arterial retention of LDL is the use of nifedipine,[238] a calcium-channel blocker that alters many cellular functions,[239] including arterial retention of autologous, but not human, LDL in normocholesterolemic rabbits.[238] Note, however, that calcium-channel blockers do not appear to affect atherogenesis in LDL receptor–deficient rabbits, which exhibit nondietary hyperlipidemia.[240] Other novel targets to consider would be inhibition of intramural production of proteoglycans or LpL, alteration of cytokine expression, such as transforming growth factor–$\beta$, and perhaps stimulation of ceramidase production.

## Concluding Remarks

Although atherosclerosis is a complex and multifactorial process, we conclude that there exists a key pathogenic event, namely, lipoprotein retention, that is both necessary and sufficient to provoke lesion initiation in an otherwise-normal artery. Other potential contributors to early atherogenesis, such as hyperlipidemia; lipoprotein influx; lipoprotein modification; turbulent blood flow; and alterations in the endothelium, smooth muscle cells, and matrix, individually fail to meet this dual criterion of necessity and sufficiency. Lipoprotein retention, however, is an absolute requirement for lesion development and appears to be sufficient in most circumstances to provoke otherwise-normal cellular and matrix elements to participate in a cascade of events leading to atherosclerosis (see the Figure). Essentially all later events can be traced to these early changes.

## Acknowledgments

Dr Williams is an Established Investigator of the American Heart Association and Genentech. During portions of this work, Dr Tabas was an Established Investigator of the American Heart Association and Boehringer-Ingelheim. Support is also acknowledged from National Institutes of Health grants HL38956, HL39703, and HL21006 and a cardiovascular research grant from the W.W. Smith Charitable Trust. We thank our colleagues for their helpful comments during the preparation of this article.

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