Site Specificity of Atherosclerosis
Site-Selective Responses to Atherosclerotic Modulators
Paul A. VanderLaan, Catherine A. Reardon, Godfrey S. Getz

Abstract—Atherosclerosis is a complex disease process that affects very specific sites of the vasculature. It is recognized that hemodynamic forces are largely responsible for dictating which vascular sites are either susceptible or resistant to developing atherosclerosis. In addition, a number of systemic and local factors also modulate the pathogenesis of the disease. By studying the development of atherosclerosis in mice, investigators have gained insights into the molecular mechanisms of this disease, although studies have largely focused on a single vascular site. Here, we review those recent studies in which vascular site-specific effects on atherosclerosis were reported when more than 1 site was examined. We assess the hypothesis that regional differences in the hemodynamic profile prime the endothelial phenotype to respond distinctly to such systemic risk factors as genetics, immune status, gender, and oxidative stress. Because a given treatment may differentially affect the development of atherosclerotic lesions throughout the vasculature, the sites chosen for study are critically important. By accounting for the complex interplay of factors that may operate at these different sites, a more complete understanding of the overriding mechanisms that control the initiation and progression of the atherosclerotic lesion may be realized. (Arterioscler Thromb Vasc Biol. 2004;24:1-11.)

Key Words: atherosclerosis • hemodynamics

Atherosclerosis is a complex chronic inflammation in the large and medium arteries that is most often associated with hyperlipidemia and/or several other risk factors. Experimentally induced animal atherosclerosis is also almost invariably associated with hyperlipidemia, although additional interventions may either attenuate or enhance lesion formation or even influence lesion composition. This chronic inflammatory response in the arteries localizes to generally reproducible sites of the vascular tree. These high-susceptibility sites are thought to be conditioned by hemodynamic parameters, being particularly associated with regions of low shear stress, oscillatory flow, or turbulent flow. These regions of disturbed flow are related to the geography of the vascular tree and are found in areas of branching or high vessel curvature. Accordingly, there are several such high-susceptibility areas for atherosclerosis. It is the goal of this review to point out that these regions may nevertheless differ from one another in their response to systemic risk factors or experimental manipulations. Hemodynamic features play a major role in the localization of atherosclerotic lesions within the vascular tree, but they are not by themselves responsible for the genesis of atherosclerosis. We postulate that hemodynamic parameters probably differ in some respects at the various high-susceptibility sites, namely, in the extent of oscillation, turbulence, or level of shear in their respective flow fields. Such regional differences may prime the local vascular wall and its gene-expression profile to differentially interact with systemic factors, thereby resulting in the potential for variations in atherosclerotic outcomes at these local sites.

To develop this hypothesis, we review briefly the limited evidence for regional differences in human atherosclerosis. We also touch on the impact of flow parameters on the various responses of the endothelium to serve as a backdrop for the consideration of potential variations that may account for regional differences in the atherosclerotic response. Because the remainder of the review focuses on murine atherosclerosis, we briefly review the major models of atherosclerosis in this species and the approaches most often used to assess the extent and size of arterial lesions. Finally, we compile the available data on site selectivity of atherosclerosis in these mouse models with a brief evaluation of the possible common mechanisms that may be the basis for this selectivity.

Regional Differences in Human Atherosclerosis
Information is limited on the extent of atherosclerosis in different regions susceptible to lesion formation, in part because multiple vascular sites are rarely analyzed in the same subject because patients generally present to their physician when their atherosclerosis disease has crossed the clinical horizon at one specific vascular bed. As a consequence, atherosclerosis in other vascular beds is then not fully
explored. Only with the advent of noninvasive methods might a more comprehensive assessment of global atherosclerotic burden be achieved. Nevertheless, there are some suggestions that atherosclerosis and its complications may exhibit selective responses in different vascular beds. Peripheral vascular disease is prominent in genetic type III hyperlipoproteinemia, a lipoprotein disorder characterized by an elevated β-migrating VLDL, enriched in apoE, that has poor receptor binding activity (typically apoE/E phenotype).1–3 Diabetes has also been shown to dramatically influence the extent and clinical complications of atherosclerosis.4 In addition to the atherosclerotic plaques frequently found in the aorta, coronaries, and carotid vessels, patients with type II diabetes mellitus have a 2- to 4-fold increase in the incidence of peripheral arterial disease, especially in the lower legs.5 Furthermore, diabetics are more likely to develop symptomatic peripheral arterial disease,6 although the basis for the relative increase of distal atherosclerosis in diabetics remains elusive.7 The carotid arteries are often unilaterally involved in atherosclerosis, with the affected artery displaying lower mean shear stress.8 Interestingly, in diabetics, the common carotid arteries have a lower mean shear stress than that found in normoglycemic individuals, with unilateral lesions again associated with localized areas of low shear stress.9 Perhaps the strongest suggestion that there is regional selectivity in atherosclerotic responses to risk factors derives from the Pathological Determination of Atherosclerosis in Youth (PDAY) study. The PDAY project represents a multicenter, autopsy-based study that examined atherosclerosis in young individuals (aged 15 to 34 years) who died of causes independent of cardiovascular disease, namely, accidents, homicides, and suicides. This represents a large group of relatively healthy subjects in whom risk factors such as hypercholesterolemia, smoking, hypertension, impaired glucose tolerance (glycohemoglobin), and gender were assessed. The effect of these risk factors on atherosclerosis was evaluated in the right coronary artery, the thoracic aorta, and the abdominal aorta. Smoking selectively increased the raised atherosclerotic burden of the abdominal aorta by 3-fold in the 25- to 34-year-old age group while not influencing right coronary artery atherosclerotic burden.10 On the other hand, elevated serum glycohemoglobin had no significant impact on lesions of the abdominal aorta but rather was associated with more extensive lesions in the right coronary artery.11 Young females had more abdominal aortic fatty streaks than did males, yet the opposite was the case for lesions found in the right coronary artery.12 Thus, it can be appreciated that different atherosclerotic risk factors affect the vasculature in unique and somewhat predictable ways, which leads to a site-selective susceptibility to the development of lesions.

**Hemodynamics and the Localization of Atherosclerosis**

It is well recognized that atherosclerosis is a focal disease manifested throughout the arterial vasculature. Differences in flow parameters within various vascular beds account for the localization of atherosclerosis, which tends to occur at sites of low shear stress, turbulence, and oscillating flow. In contrast, areas of laminar flow are relatively resistant to the development of early atherosclerosis. We do not here contend that hemodynamic parameters are by themselves causative of atherosclerosis but rather that they “prime the soil” in which lesions develop. There are numerous reproducible sites that are prone to developing atherosclerosis. Among these sites are those that exhibit high susceptibility, whereas others may exhibit only moderate susceptibility.13 For example, Sims14 has studied the elastic lamina in the human coronary artery and the internal mammary artery, highly atherosclerosis-susceptible and atherosclerosis-resistant arteries, respectively. In the latter, the internal elastic lamina is closely applied to the endothelium without notable discontinuities. This is in contrast to the coronary arteries of these same subjects, in which lipids and macrophages accumulate in fragmented regions of the elastic lamina. Whether these findings are attributable to hemodynamic differences alone or to variations in the inherent properties of these vessels is not yet clear.

There are overwhelming correlative data that indicate that low shear or disrupted flow accounts for the localization of atherosclerosis. Perhaps most striking is the pathological observation that within a given cross section of a susceptible site, the initial lesion is almost always eccentrically distributed in a predictable fashion. For example, at the branch points in the innominate artery or the iliac bifurcation, the initial lesion is found on the lateral wall. The reason for this specific localization can be explained by local hemodynamics, as explored below.

Caro et al15 in 1969 pointed to the important correlation between low shear stress and atherosclerosis in the vasculature. This concept was further elaborated by Glagov and colleagues.16,17 By reproducing flow conditions in scale human carotid bifurcation models, they showed that the localization of atherosclerosis found at autopsy correlated to areas of flow disruption, which led to increased particle residence time.16,17 Similar modeling was performed on the abdominal aorta, in which both oscillating flow and low shear stress were observed along the posterior wall of the infrarenal aorta, where atherosclerotic lesions develop.18 Pres lesion areas that are susceptible to the development of atherosclerosis may be differentiated from other less susceptible areas. In young subjects, nonatherosclerotic intimal thickening is more likely to be found in regions of the aorta that are prone to develop atherosclerosis.13,19 Fetal fatty streaks are found in the presence of maternal hypercholesterolemia, and these lesions again localize to areas susceptible to developing atherosclerotic plaques.20 Furthermore, studies in mice have shown that maternal hypercholesterolemia during pregnancy, much as hemodynamics, appears to prime the gene expression of the vessel wall in pups, which persists even after the hypercholesterolemia is reversed.21 This vascular diversity with respect to atherosclerotic susceptibility is also seen in large animals frequently used for atherosclerosis work, namely, pigs, dogs, and rabbits. In these mammals, there is evidence of increased permeability in susceptible areas of the aorta before lesion development that correlates with future sites of lesion development. This heterogeneity of local vessel permeability and its association with differential susceptibility to atherosclerosis was first
noted by McGill and colleagues using Evans blue dye (bound to albumin) and was further developed by Bell and colleagues. Albumin-bound Evans blue dye, radioactive albumin, radioactive fibrinogen, horseradish peroxidase, and radioactive LDL have all been shown to localize to areas of increased permeability and increased atherosclerosis susceptibility. In general, there is a gradient of aortic permeability to LDL in a cephalic to caudal direction, and this is mirrored by cholesterol accumulation in the aorta of cholesterol-fed rabbits. At the rabbit aortoiliac bifurcation, LDL incorporation is greater along the lateral wall, with lower shear force and higher susceptibility for atherosclerosis than along the medial wall. Finer mapping of this enhanced permeability is seen in the normal rabbit aorta, measured with macromolecular horseradish peroxidase. The distribution of permeability “hot spots” was reproducible, generally decreasing distally, with a minimum in the lower thoracic aorta, but increasing again in the abdominal aorta. The density of these spots is high in the distal region of aortic ostia and is consistent with an influence of hemodynamic phenomena. One needs to bear in mind that the measurement of increased permeability may actually reflect increased lipoprotein retention, mediated by subendothelial matrix proteoglycans at these sites of apparently higher permeability.

These aforementioned hemodynamic phenomena are perceived by the endothelial cell, and the potential sensing mechanisms have already been reviewed extensively by Davies and colleagues. Endothelial cells are known to align with the axis of laminar flow. This alignment is abolished in areas of disturbed flow, even over a small spatial scale that represents only a few cells. The flow-related response is transmitted to the intracellular cytoskeletal filaments that connect to organelle and nuclear membranes. This distribution of luminal signal potentially results in an integrated cellular response, which involves several cell-surface and intracellular-signaling molecules, without implicating a single primary biomechanical sensor.

The endothelium, as the cell type that senses mechanical forces in the vasculature, has been widely studied in tissue culture under conditions of carefully defined flow environments. These studies have focused on the responses of genes in cells exposed to laminar flow, turbulent flow, or oscillating flow. Several important studies have recently subjected such cells to gene profiling. Notably, these gene-profiling studies differ in a number of respects, including the origin of the endothelial cells used, the flow profiles to which they were subjected, and the specific microarrays and methodology used for expression analysis. Thus, it is not surprising that there is only a moderate degree of concordance in gene expression in response to shear. There are a variety of responses among gene groups, with some genes exhibiting reciprocal responses to laminar and disturbed flow and others reacting to only 1 of the flow parameters. These responses involve both upregulation and downregulation. An important example is illustrated by the experiments of Garcia-Cardena and colleagues, who showed that at least 100 genes are differentially regulated between endothelial cells when turbulent shear is compared with laminar shear, 68 being upregulated and 32 being downregulated. The biological meaning of this differential gene expression is yet to be fully elucidated. This said, there are several groups of gene products that are worthy of more detailed consideration, such as cell-surface adhesion molecules (vascular cell adhesion molecule-1 [VCAM-1] and intracellular adhesion molecule-1 [ICAM-1]), pro-oxidant enzymes (lipoxygenases, NADPH oxidases), and antioxidant enzymes (NO synthase, superoxide dismutase). Each of these gene families has displayed some degree of flow responsiveness. Additionally, it has been shown that flow conditions may influence the maturation of the sterol regulatory element binding proteins (SREBP). A particularly interesting target of SREBP, short-chain aldehyde reductase, has been reported recently. This enzyme, if acting in an antioxidant fashion, may protect the vessel wall from the effects of oxidized fatty acids.

Oscillatory shear stress substantially upregulates VCAM-1, ICAM-1, and E-selectin in cultured human endothelial cells. Interestingly, the expression of these molecules was also upregulated under static conditions when endothelial cells were cocultured with smooth muscle cells, yet this inducing effect of the smooth muscle cells was inhibited by applied shear stress. These changes in gene expression are mediated by nuclear factor-κB (NF-κB) activation, which occurs particularly under low shear conditions. NF-κB activation is associated with a reduction in eNOS in a flow-dependent fashion, which in turn is related to eNOS kinase activation. The in vivo significance of these findings in cultured endothelial cells is mirrored by the high level of expression of NF-κB in the lesser curvature of the murine aorta, which has a disrupted flow pattern and is susceptible to atherosclerosis. This contrasts with the more restricted expression of NF-κB in the greater curvature, which has a more laminar flow and is relatively resistant to atherosclerosis. Overexpression of endothelial cell NO synthase (eNOS) inhibited NF-κB activation induced by low shear. eNOS is induced in a dose-dependent manner by laminar shear stress, whereas oscillatory shear stress can downregulate eNOS. This activation of eNOS is largely mediated by specific protein kinase B–dependent phosphorylation. One of the earliest responses to hypercholesterolemia is the attenuation of endothelium-dependent arterial relaxation and production of NO. A decrease of eNOS has been noted in the atherosclerosis-susceptible areas of the aorta in LDL receptor–deficient mice, which suggests that NO production may be protective against the development of atherosclerosis. eNOS is predominantly located in caveolae, and this localization is reduced by oxidized lipoprotein and increased by HDL in an SR-BI–dependent fashion. Importantly, the cholesterol content of caveolae may influence endothelial cell signaling. The cholesterol content could be regulated in part by endogenous synthesis mediated by the SREBP pathway, which again is activated by shear stress.

There is increasing evidence that reactive oxygen species may be strong proatherogenic mediators. The most important contributor to increased reactive oxygen species is NADPH oxidase, which is regulated differentially by laminar and oscillatory shear stress, the latter being responsible for the
activation of this oxidase. The increased NADPH oxidase activity may be posttranscriptional, depending on phosphorylation of its subunits. There are many agonists for this oxidase, including angiotensin II. Other members of the reactive oxygen species system that are influenced by flow parameters include heme oxygenase and Cu/Zn superoxide dismutase. It is clear that the balance between pro-oxidant and antioxidant elements may determine the likelihood of developing atherosclerosis at a particular vascular site and that this balance is influenced by shear stress magnitude and oscillation. This notion serves as a prototype for the balancing of proatherogenic and antiatherogenic influences. Other balances among adhesion molecules, chemoattractant molecules, cytokines, growth factors, and survival factors all potentially play a role in either promoting or inhibiting the process of atherosclerosis at any given vascular site.

Murine Atherosclerosis

Mouse Models

Although the findings obtained with cultured endothelial cells provide valuable insights into the types of in vitro flow-related regulation that these genes and proteins exhibit, these experiments cannot accurately replicate the in vivo situation, especially if the possibility of regional variations and the average consistency of hemodynamics over an extended period of time are taken into account. Therefore, effective in vivo models are necessary.

Because of the obvious difficulties in studying pathogenic mechanisms in individual human subjects, a well-characterized experimental model of atherosclerosis is mandatory. Over the past decade, the mouse has emerged as the best model because of its rapid reproduction, the extensive knowledge of its genetics, the ability to manipulate its gene expression, the relatively rapid lesion formation in genetically modified mice, and the relative ease of lesion analysis. Unfortunately, the ability to study hemodynamics in the mouse is difficult because of size constraints. This reservation notwithstanding, an improved understanding of early atherogenesis has emerged in recent years based primarily on the mouse model (reviewed in Lusis and Glass and Witzum).

Mouse models of atherosclerosis present the opportunity to explore the site preferences for lesion development under the influence of a variety of risk factors and modulators of atherogenesis. A number of recent reviews have extensively discussed the different mouse models used to study atherosclerosis. As alluded to earlier, the development of atherosclerotic lesions in the vasculature of mice also occurs at very reproducible sites that are consistent with being determined predominately by the hemodynamic forces experienced by the endothelium. This vascular distribution is
However, a few mouse models have been generated that lead to bleeding and thrombosis have been reported in mice, examples of complicated lesions with plaque disruption. The aortic sinus is not characteristically involved in human atherosclerosis. The very rapid heart rate (550 bpm in the mouse compared with 70 bpm in the average human) may account for this difference. Because flow in the murine aortic sinus is likely to be much more disrupted than in the human aortic sinus. Along these lines, Bassiony and colleagues have shown that heart rate is an important determinant of atherogenesis.

To appreciate the potential for selective modulation of atherosclerosis at various vascular sites at risk, it is necessary to study at least 2 such sites, which is only now beginning to be appreciated. The way a lesion responds to the most commonly studied sites is not necessarily reflective of pathogenesis at other sites. Indeed, by focusing only on the aorta or aortic sinus, effective modeling of unstable advanced plaque remained elusive until researchers began looking elsewhere, namely, the innominate artery.

The most important sites for clinically significant atherosclerotic disease in humans are the coronary arteries, with progression to atherothrombotic events and subsequent myocardial infarction. Few mouse models reproducibly develop clinically relevant lesions in the myocardial coronary arteries, although in some older LDL receptor- and apoE-deficient mice, examples of complicated lesions with plaque disruptions leading to bleeding and thrombosis have been reported. However, a few mouse models have been generated that do lead to widespread coronary artery atherosclerosis. These include the SR-BI/apoE double-knockout mouse that recently has been shown to spontaneously develop myocardial infarctions and cardiac dysfunction as a result of extensive lipid-rich coronary artery occlusions and the apoE/LDL receptor double-knockout mouse fed a Western-type diet that has similar coronary artery atherosclerosis and stress-induced myocardial infarction. In addition, advances in microangiography have facilitated the detection of coronary artery stenosis in apoE-deficient mice. Finally, the atherosclerotic plaques that develop in the murine innominate artery, left common carotid, and left subclavian branches of the aorta, although potentially more difficult to access and sometimes requiring longer for lesions to develop, more closely mimic plaques found at the human carotid bifurcation and therefore deserve further attention.

With respect to the local hemodynamic environment that influences murine atherosclerosis, the circumferential asymmetry of atherothrombotic lesions in both the mouse aortic sinus and innominate artery are shown in Figure 2. As typified here, the lesion found in the innominate artery is always localized to the lateral wall, and a greater amount of lesion develops on the aortic sinus proximal to the lesser curvature of the aorta than the greater curvature side. Once again, these sites are precisely where turbulent, pulsatile, and nonlaminar flow forces predominate, promoting the development of atherosclerosis.

**Measuring Atherosclerosis**

As stated previously, to study atherosclerosis site specificity, analysis of more than 1 site is required. In the investigations that have examined different vascular sites, the tendency has been to perform cross-sectional analysis of the aortic sinus in conjunction with measuring the surface area of atherosclerotic plaques in Sudan IV–stained aorta by en face analysis. Although this practice provides 2 independent assessments of atherosclerosis for each mouse, it is still preferable to use the same methodology when 2 or more sites are being examined if site-specific effects are to be truly appreciated. Although Tangirala et al demonstrated a strong correlation between these 2 assay methods in 1 mouse model, this is not always the case. Furthermore, these 2 methods provide very different types of information about the lesion. The en face method, although providing an aggregate measure of the extent of the aorta covered by lesion, provides little information on lesion thickness, its cellular composition, or the possibility that select areas of the aortic surface may exhibit a bias in the development of lesion unless a careful delineation of specified aortic regions is attempted. These reservations notwithstanding, by dividing the aorta into distinct zones, it is possible to see regional variations in plaque burden. This is exemplified in the study by Lichtman et al, who showed that cholate in the diet selectively influenced thoracic aortic atherosclerosis as measured by en face methodology. Other methods have also been used for analysis of atherosclerosis. A simple and reliable quantitative global measure of atherosclerosis is obtained by the biochemical measurement of the free cholesterol and cholesterol ester content isolated from the entire aorta. Unfortunately, this technique reveals...
little about the distribution, localization, or morphology of individual lesions. The use of magnetic resonance microscopy has the advantage of imaging atherosclerotic plaques in vivo, correlating well with traditional histopathological measurements, and enabling the investigator to monitor lesion development within individual animals over time.80–82 Unlike the carefully selected cross-sectional analysis, most of these measurement methods lose much of the information content in lesion distribution.

Although vascular cross-sectional analysis satisfies the need for information on cellular composition, it is a quantitative assessment that is very sensitive to precisely where the lesions are sectioned and is more time consuming than the en face measurement. Nevertheless, much of our current understanding of atherogenesis has been derived from cross-sectional analysis of the aortic sinus. Cross-sectional analysis is probably the only method that provides significant information on lesion progression and initiation, because it is more likely to reveal information on lesion complexity that relates to lesion progression. Methods that rely on lesion extent may not reveal much information on progression. Ideally, models are needed that can separate lesion initiation from lesion progression, which is not easy to envisage because lesion growth results at least in part from initiation of type responses at the lesion shoulder. Most studies of murine atherosclerosis do not differentiate fatty streaks from more complex lesions, reflecting progression-type responses. Appreciation for the complexity of the pathogenesis of atherosclerosis and the richness of its regulation will be facilitated by the extension of these focused morphological studies to other sites as well.

Finally, when one studies regional variations in atherosclerosis, it is important to bear in mind the rate of lesion development at each site. The sampling time for atherosclerosis measurement can easily influence the reported outcome. For example, with 15-lipoxygenase overexpression,83 aortic sinus lesion area is greatly increased when examined in mice after 3 or 6 weeks of feeding a high-fat atherogenic diet but not after 9 weeks. At a more refined level, cellular lesion composition can also change over time. For example, smooth muscle cell content is markedly reduced in the aortic arch lesions of interferon (IFN)-γ/LDL receptor double-deficient mice when sampled after 8 weeks on diet but not after 20 weeks on diet.44 Therefore, it is important to appreciate that the complexity of the atherosclerotic lesion might not necessarily be captured in a given assessment, and mechanistic conclusions must be made with these limitations in mind.

**Site Specificity of Murine Atherosclerosis**

The preceding discussion has reviewed some of the evidence that the localization of atherosclerotic plaques is largely determined by flow patterns. Furthermore, we have reviewed some of the evidence that disturbed flow patterns influence the expression of a large number of endothelial cell genes, which in turn have the capacity to promote lipoprotein oxidation, the redox state, and the adhesion of inflammatory cells that participate centrally in atherogenesis. The flow patterns to which endothelial cells are exposed for the lifetime of the animal may prime the gene-expression profile in endothelial cells at particular locations, either promoting or attenuating the development of atherosclerosis at these sites. This is exemplified in the activation of NF-κB specifically in the lesser curvature of the mouse aorta, an atherosclerosis-susceptible area, by diet-induced hypercholesterolemia and lipopolysaccharide injection.44 It is evident that hemodynamic factors are not by themselves sufficient to induce atherosclerosis, for some mouse strains (such as C3H or FVB) are quite resistant to atherosclerosis. Although not categorically tested, it is highly unlikely that the sensitive and resistant strains differ in their hemodynamic patterns. Endothelial cells have been suggested to be the locus of resistance in C3H mice, markedly altering the initial stages of atherosclerosis.85 Thus, even though the potentially susceptible sites are probably subject to similar flow patterns in resistant strains, it is possible that the inherent phenotype of the barrier endothelial cell precludes the evolution of the atherogenic pathway.

There are few studies that allow one to specifically address the question of the site-selective evolution of atherosclerosis. Several of the experiments in mouse models, in which the response varies by vascular region, are summarized in the Table. It can be expected that future work will uncover many such instances, and these differences may illuminate the richness of atherogenic mechanisms.

Given that atherosclerosis develops at sites of low shear and disturbed flow, we propose that the flow patterns in different regions vary in quantitative detail and that these variations have a complex effect on the development of atherosclerosis. These variations may influence the relative residence time of lipoproteins, blood-borne molecules, and inflammatory cells that come into contact with the endothelial cells in each of these regions. Also, the hemodynamic patterns may prime the gene-expression profile of endothelial cells in subtly different ways so that these cells react to global risk factors such as hyperlipidemia, gender, and the immune system in regionally distinct fashions. Tissue culture studies, although clearly indicating the flow responsiveness of endothelial genes, do not accurately model the fine details of the in vivo situation that bears on site-selective atherosclerosis. Only in vivo sampling of particular vascular regions for detailed comparative studies is likely to yield critical information on these questions.

To this point, most of the in vitro work on the influence of flow patterns on endothelial cell gene expression has used cells either derived from the human umbilical vein (a site not susceptible to atherosclerosis) or from human, bovine, or rabbit aortas. In no instance have endothelial cells for the study of gene expression been derived from different regions of the vascular tree that are both prone to developing atherosclerosis, albeit at different rates or under different circumstances. Such a study could potentially indicate whether endothelial cells exhibit a regionally specific phenotype that would account for atherosclerotic site selectivity.

This said, even cultured endothelial cell studies have pointed to the balance between genes that promote or impede atherogenesis that are candidates for study of regional in vivo differences. What has been shown is that some of the flow-responsive genes vary as the magnitude of the flow parameter is altered (VCAM-1,101 for example), which makes such genes potential candidates for regional variation in
atherosclerosis. The following are some of the other factors that could influence or appear to influence regionally distinct lesion development.

**Age of Lesion**

Because the initiation and rate of lesion progression may vary between different sites, the age of the atherosclerotic lesion needs to be considered, especially when one examines animals at a single time point after an experimental manipulation. When analysis is confined to a single time point, the oldest lesion will be the one initiated first, assuming a similar rate of lesion progression at the 2 sites. In murine models, the aortic sinus lesion is the earliest one to develop. Thus, manipulations that influence lesion initiation would be expected to have the greatest effect on atherosclerosis at this site, especially if assessed shortly after application of the intervention and before the lesion progresses substantially. On the other hand, with experimental intervention after the aortic sinus atherosclerosis is initiated or allowed to progress, lesions in the rest of the vasculature that were initiated later than the aortic sinus lesion may be more affected. For example, this could account for the results seen in 12/15-lipoxygenase/ apoE103 and 12/15-lipoxygenase/LDL receptor92 double-knockout mice in which lesions in the aorta were reduced to a greater extent than lesions in the aortic sinus. The influence of lipoxygenase deficiency on the proximal aorta and its branches is evident even at 10 weeks of age. It is inferred that 12/15-lipoxygenase acts to promote lesion initiation by increasing the oxidative burden in the early plaque. The early stages of atherogenesis are evident when the enzyme is overexpressed in the endothelium of LDL receptor–deficient mice. On the other hand, the effects of immune deficiency on aortic sinus and innominate artery lesions of apoE–deficient mice73 and LDL receptor–deficient mice69 could not be accounted for on this basis, because morphologically advanced innominate artery lesions are not influenced by immune deficiency. Although it is unlikely that many of the site-specific effects listed in the Table can be explained solely on the basis of the time or age at which lesions were sampled, the above considerations suggest the advisability of more than 1 sampling time when atherosclerosis is studied at multiple sites.

**Oxidation Profile**

The oxidative modifications of lipids retained in the vessel wall are regarded as early and critical events in the development of atherosclerosis57,58; furthermore, oxidative events independent of lipid modification likely play a role in atherogenesis. By preventing lipoprotein oxidation with antioxidants, early events in the generation of the atherosclerotic lesion should be blocked, thereby halting initiation of the lesion. At least 5 of the 17 studies cited in the Table are involved directly or indirectly with oxidative systems. Three antioxidants (vitamin E, CoQ10, and probucol) have been used with quite variable results on the relative extent of lesions in the aortic sinus and the rest of the aorta. On the one hand,
reduction in specific oxidation pathways through elimination of either 12/15 lipoxygenase or the p47 NADPH oxidase subunit in the apoE-deficient background reduces aortic atherosclerosis to a greater extent than that in the aortic sinus. A similar mechanism may account for the results of the interleukin-4 deficiency study, because interleukin-4 regulates 12/15 lipoxygenase. On the other hand, oxidized LDL immunization has an opposite influence on the relative distribution of lesions between the aortic sinus and the rest of the aorta. Therefore, it is evident that manipulation of the oxidative status of the early plaque may have divergent results with respect to overall atherosclerosis that are dependent on both the timing and the specific oxidative pathway modified.

Genetic Background

The above site-specific effects have been studied in either apoE-deficient or LDL receptor−deficient mice, most often backcrossed into the C57BL/6 background. Among the variety of inbred mouse strains, there is large variation in the incidence and extent of atherosclerosis at the aortic sinus even in the context of apoE or LDL receptor deficiency. Studies of strain differences in atherosclerosis susceptibility (for example, FVB) have focused largely on the aortic sinus lesion. This could bias the selection of potentially influential genes on atherosclerosis that operate primarily at this site, whereas other sites may be affected by a different set of modulator genes. We have preliminary data that variation in innominate artery atherosclerosis may indeed be regulated by a gene or genes that are not quite so relevant in aortic sinus lesion formation. At any rate, the specific genetic background of the murine model used is an important caveat that needs to be considered in the analysis of genes that affect the extent of the atherosclerotic lesion.

Gender-Specific Effects

The gender-related differences in the development of atherosclerosis are of great clinical interest. Estrogen administration has been shown to have a number of proatheroprotective effects in murine models. However, in contrast, both apoE-deficient and LDL receptor−deficient female mice generally develop larger lesions than males at the aortic sinus (C.A. Reardon et al, unpublished data). Early characterization of lesion distribution throughout the rest of the aorta in both LDL receptor−deficient and apoE-deficient mice has highlighted subtle gender-dependent differences in the distribution of atherosclerotic burden throughout the vasculature. Administration of the peroxisome proliferator−activated receptor-γ agonist rosiglitazone to LDL receptor−deficient mice results in selective lowering of atherosclerosis only in male mice. There is also an interaction between gender and immune deficiency in the site-specific effects on the development of atherosclerosis. The superimposed effect of IFN-γ deficiency on apoE deficiency results in a reduction of atherosclerosis in male but not female mice, and this correlates with the abundance of T cells and cells expressing major histocompatibility complex class II. Global immune deficiency reduces aortic sinus atherosclerosis in both male and female apoE-deficient mice and in LDL receptor−deficient mice fed a Western-type diet (Reardon et al, unpublished data, and References 73, 89, 116, and 117). Male and female immune-competent apoE-deficient mice have similar levels of innominate artery atherosclerosis, but immune deficiency reduces innominate artery atherosclerosis in female but not male apoE-deficient mice. A complex gender-specific effect of immune status is observed in apoE-deficient mice. In these mice, immune-competent females have significantly less innominate artery atherosclerosis than males, but the amount of innominate artery atherosclerosis in immune-deficient female and male mice is comparable (Reardon et al, unpublished data). It is unlikely that these complex effects of immune deficiency are attributable simply to the reduced production of IFN-γ, because IFN-γ deficiency has no measurable effect on atherosclerosis in female apoE-deficient mice.

Conclusions

It is clear from the information reviewed above that like atherosclerosis itself, the variation of lesion development at different sites is sensitive to several parameters. These include genetic background, immune status, gender, and oxidative stress, each of which may differentially influence atherosclerosis at distinct sites. These various influences on site-specific modulation of atherosclerosis are conceptualized in Figure 3, using the aortic sinus and innominate artery as examples. Here, we illustrate the notion that hemodynamic parameters differentially prime the genetic substrate at each vascular atherosclerosis-susceptible site, allowing for a distinct response to 1 or more of the systemic risk factors that might be at play in each particular circumstance. We do not suggest that atherogenic mechanisms fundamentally differ at these distinct sites but rather that these mechanisms interact with modulatory influences in complex ways to generate the lesion at a particular site. We propose that the blood flow profile and flow-disruption pattern uniquely and quantitatively prime the gene-expression response at each of these risk sites (even in the absence of atherosclerosis), and that these in turn interact with the many systemic factors that engender or attenuate atherosclerosis to provide the integrated response that we see as a lesion of a particular size and character. At the moment, no clear pattern of modulating factors emerges to account for the varied atherosclerotic responses at select sites of the vascular tree. Hopefully, the advent of extensive gene profiling and novel imaging approaches will help to unravel these complex interactions, or at least furnish a deeper understanding of this intricate disease process. In particular, it is expected that future studies of multiple regions susceptible to atherosclerosis in a variety of animal models will further illuminate both the subtleties and complexities of atherogenic mechanisms in the pathogenesis of this disease process.

Acknowledgments

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Figure 3. Model for conceptualizing the interplay between numerous factors that result in site-specific modulation of atherosclerosis. The local hemodynamic flow patterns at 2 atherosclerosis-susceptible sites exemplified by the aortic sinus and innominate result in the differential gene-expression profiles of the vascular cells at each of these sites. The specific responses of these primed vascular cells to the risk factors and modulators of atherosclerosis will affect the atherogenic pathways, which in turn could result in differences in the size, composition, or rate of progression for each atherosclerotic lesion. On the basis of our murine atherosclerosis studies in male mice, we hypothesize that the primed cells of the aortic sinus are more susceptible to immune deficiency than are cells in the innominate artery, whereas genetic background influences atherosclerosis at both sites.

1. The local hemodynamic flow patterns at 2 atherosclerosis-susceptible sites exemplified by the aortic sinus and innominate result in the differential gene-expression profiles of the vascular cells at each of these sites. The specific responses of these primed vascular cells to the risk factors and modulators of atherosclerosis will affect the atherogenic pathways, which in turn could result in differences in the size, composition, or rate of progression for each atherosclerotic lesion. On the basis of our murine atherosclerosis studies in male mice, we hypothesize that the primed cells of the aortic sinus are more susceptible to immune deficiency than are cells in the innominate artery, whereas genetic background influences atherosclerosis at both sites.


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