Janus Phenomenon
The Interrelated Tradeoffs Inherent in Therapies Designed to Enhance Collateral Formation and Those Designed to Inhibit Atherogenesis

Stephen E. Epstein, MD; Eugenio Stabile, MD, FESC; Timothy Kinnaird, MD; Cheol Whan Lee, MD; Leonardo Clavijo, MD; Mary Susan Burnett, PhD

Enormous advances have occurred in the understanding of the molecular and cellular mechanisms responsible for both collateral development (collaterogenesis*) and atherogenesis. This advancement has been accompanied by parallel advances in therapies designed to enhance collaterals and to inhibit the development and progression of atherosclerosis. Our laboratory has been interested in both areas, and during our work, we noticed a consistent tradeoff: Whatever intervention enhanced collaterals increased atherogenesis and visa versa. We refer to this tradeoff as the Janus phenomenon.† This observation, if correct, is of more than passing interest given the many antiatherosclerotic interventions used in patients and the proangiogenic interventions being tested that may soon be used therapeutically. Therefore, if the Janus phenomenon is real, its clinical implications would be of critical importance. Equally as important, the concept might provide mechanistic insights into both atherogenesis and collaterogenesis.

Consequently, we began to track phenomena compatible with the Janus concept. Table 1 lists a few molecules for which reasonable evidence exists relating to both their atherosclerotic and collaterogenic effects and the actions of which are compatible with the Janus phenomenon.† Complementary to the information contained in Table 1 are the opposite concordances displayed by different mouse strains regarding collaterogenic potential versus atherogenic susceptibility. C57BL/6 mice are susceptible to atherosclerosis and are exuberant collateral formers. Conversely, BalbC mice are resistant to atherosclerosis and have an inferior collaterogenic capacity,19,20 suggesting that genetic determinants conveying susceptibility to atherogenesis will convey an enhanced capacity for collaterogenesis and visa versa. Therefore, the Janus phenomenon, expressing concordance between atherogenic and angiogenic proclivities, appears also to apply to genetic predispositions. (However, just the opposite properties in the neointimal response to acute vascular injury have been observed between C57Bl/6 and BalbC mice—BalbC>C57BL/6.21 It is therefore uncertain whether the Janus phenomenon holds for genetic propensities to develop restenosis.) Such concordances indicate that shared mechanisms contribute to collaterogenesis and atherogenesis, that stimulating these mechanisms will activate both processes, and conversely that inhibiting these mechanisms will inhibit both processes. The concept (the Figure) therefore lends itself to posing and testing new mechanistic hypotheses. Applying what we know about molecular mechanisms of atherogenesis to collaterogenesis might provide mechanistic insights about collaterogenesis that would otherwise go unsuspected and visa versa. Also, determining the molecular basis for the different capacities of C57BL/6 and BalbC mice to develop collaterals will probably shed light on molecular mechanisms contributing to atherogenesis.

Inflammation in Atherogenesis and in Collaterogenesis

The processes involved in atherogenesis and collaterogenesis appear to have little in common. However, there is overlap, and one of the more important shared mechanisms involves inflammatory responses.22,23 (the Figure). Thus, atherosclerosis is an inflammatory disease triggered and sustained by inflammation-related cytokines, chemokines, and adhesion molecules and by the cellular components of the immune system such as monocytes/macrophages and T lymphocytes, which express such molecules.23 Compelling data suggest that these same cells and molecules also contribute importantly to collaterogenesis.24 In particular, macrophages are central to arterial remodeling in tissues in which collaterals are forming. These cells accumulate at sites of collateral growth and are an important source of vascular endothelial...
growth factor, tumor necrosis factor-α, and basic fibroblast growth factor, each of which contribute to collaterogenesis. Moreover, the absence of macrophages is associated with deficient collaterogenesis.

A role of T lymphocytes in collaterogenesis was suggested by the impaired collateral response to hindlimb ischemia observed in athymic mice. More recently, it was demonstrated in a mouse model of hindlimb ischemia that CD4+ T lymphocytes are recruited to sites of collateral vessel remodeling, secrete multiple cytokines, and influence the trafficking of other cellular components of the immune system (ie, macrophages), and when absent (in a knockout model), collaterogenesis is significantly impaired.

These and other studies also suggest that tissue ischemia induces a systemic inflammatory response. Intuitively, although such a response might enhance collaterogenesis, it would also contribute to inflammatory processes already present in the arterial wall of atherosclerotic arteries and thereby exacerbate atherogenesis.

Both collaterogenesis and atherogenesis are complex processes involving hundreds of different molecular and cellular regulators. DNA array expression profiling, which allows evaluation of the activation of thousands of genes in experimental settings modeling these processes, can enhance our understanding of their differences and similarities. In fact, when the femoral artery of C57BL/6 mice is ligated to induce peripheral ischemia and collateral formation, many of the pathways common to atherogenesis and collaterogenesis underlying the Janus phenomenon. Atherogenesis, ischemia, and increased shear stress in developing collaterals activate multiple genes, many related to inflammation. Expressed cytokines exert local effects but also are released systematically, activating immune and inflammatory cells located in such tissues as bone marrow and spleen. These cells home to activated tissues and contribute to collaterogenesis or atherogenesis. Given these common mechanisms, the Janus phenomenon is based on the assumption that an intervention altering any component of these multiple pathways will exert a corresponding effect on both end processes: atherogenesis and collaterogenesis. VEGF indicates vascular endothelial growth factor; MCP, monocyte chemotactic protein; and PPARγ, peroxisome proliferator–activated receptor-γ.

### Table 1. Molecules With Activities Compatible With the Janus Phenomenon

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Atherogenesis</th>
<th>Collaterogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>Exacerbates1</td>
<td>Enhances1–4</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Exacerbates2,6</td>
<td>Enhances2,6</td>
</tr>
<tr>
<td>FGF-1</td>
<td>Exacerbates3,7</td>
<td>Enhances3,9</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Exacerbates4,7</td>
<td>Enhances4,7</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Inhibits11–13</td>
<td>Inhibits14</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Exacerbates5,7</td>
<td>Enhances5,16</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Inhibits12</td>
<td>Inhibits12</td>
</tr>
</tbody>
</table>

VEGF indicates vascular endothelial growth factor; FGF, fibroblast growth factor; MCP, monocyte chemotactic protein; and PPARγ, peroxisome proliferator–activated receptor-γ.
genes upregulated at the site of collateral development also causally relate to atherogenesis (Table 2).

One example of a molecule exerting such Janus-like activities is the chemokine monocyte chemoattractant protein (MCP-1). MCP-1 recruits monocytes and is one of the critical mediators of atherogenesis. It also plays a role in collaterogenesis, an effect mediated through its recruitment of monocytes. Further confirmation of the dual Janus-compatible effects of MCP-1, which would be anticipated given the role of the monocyte/macrophage in both atherogenesis and collaterogenesis, is the demonstration that infusing MCP-1 simultaneously enhances collaterogenesis in the ischemic hindlimb of apolipoprotein E (apoE)–knockout mice, which differential gene expression was assayed in the region of developing collaterals after femoral artery ligation of the mouse hindlimb showed increased expression.

Although evidence indicates a role of proinflammatory cytokines in collaterogenesis and atherogenesis, the role of antiinflammatory molecules in both these processes is uncertain. Among antiinflammatory cytokines, IL-10 has putative antiangiogenic and antiatherogenic effects. For example, when absent, the inflammatory response to peripheral ischemia is exuberant, and collateral development is triggered. Similarly, IL-10 deficiency increases atherosclerosis. These data suggest that IL-10 negatively regulates atherogenesis and collaterogenesis.

**Metabolic Factors**

Factors associated with the metabolic syndrome such as the adipocyte-expressed molecules leptin and adiponectin also appear capable of exerting Janus-like effects. Thus, leptin has proangiogenic effects, and it has been suggested that the increased levels of this molecule found in obesity is one of the mechanisms whereby obesity predisposes to atherosclerosis. Conversely, adiponectin suppresses the development of atherosclerosis but, in Janus-like form, exerts antiangiogenic effects.

**Progenitor Cells in Atherogenesis and in Collaterogenesis**

Recent experimental studies demonstrated a remarkable collaterogenic effect of bone marrow–derived progenitor cells, results that have led to several clinical trials. However, the Janus phenomenon implies that these cells might also make atherosclerosis worse. Indeed, although

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**TABLE 2. Partial List of Cytokines/Chemokines and Metabolic Factors Identified as Being Involved in Atherogenesis and Possible Relation to Collaterogenesis**

<table>
<thead>
<tr>
<th>Adhesion/Cytokine/Chemokine</th>
<th>Atherogenesis</th>
<th>Collaterogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>Increases</td>
<td>Likely increases*</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Increases</td>
<td>Likely increases*</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Increases</td>
<td>Likely increases*</td>
</tr>
<tr>
<td>Chemokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (monocyte chemoattractant)</td>
<td>Increases</td>
<td>Increases</td>
</tr>
<tr>
<td>IP-10 (lymphocyte chemoattractant)</td>
<td>Probably increases</td>
<td>Expression increases</td>
</tr>
<tr>
<td>MIG (lymphocyte chemoattractant)</td>
<td>Probably increases</td>
<td>Expression increases</td>
</tr>
<tr>
<td>Cytokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (induces MCP-1, IP-10, and MIG, and probably has other effects)</td>
<td>Increases</td>
<td>Possibly increases*</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Probably increases</td>
<td>Expression increases</td>
</tr>
<tr>
<td>IL-6</td>
<td>Probably increases</td>
<td>Expression increases</td>
</tr>
<tr>
<td>IL-10</td>
<td>Inhibits</td>
<td>Inhibits</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Increases</td>
<td>Increases</td>
</tr>
<tr>
<td>Metabolic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>Possibly increases</td>
<td>Possibly increases</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Decreases</td>
<td>Possibly decreases</td>
</tr>
</tbody>
</table>

VCAM indicates vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; MCP, monocyte chemoattractant protein; IP-10, interferon-inducible protein 10; MIG-1, monokine induced by interferon-γ; and TNF, tumor necrosis factor. Expression increased means that DNA array study in which differential gene expression was assayed in the region of developing collaterals after femoral artery ligation of the mouse hindlimb showed increased expression.

*Likely, at least through recruitment of monocytes or T lymphocytes (direct proof lacking).
TABLE 3. Apparent Janus-Like Effects of Cells on Atherogenesis and Collaterogenesis

<table>
<thead>
<tr>
<th></th>
<th>Atherogenesis</th>
<th>Collaterogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-derived MNC</td>
<td>Increases,76</td>
<td>Inhibits77</td>
</tr>
<tr>
<td>Monocyte/macrophage</td>
<td>Increases53,72</td>
<td>Increases10</td>
</tr>
<tr>
<td>T cells</td>
<td>Increases23,53</td>
<td>Increases23</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Increases53,74</td>
<td>Increases49</td>
</tr>
</tbody>
</table>

BM-derived MNC indicates bone marrow-derived mononuclear cells.

intravenous injection of bone marrow–derived mononuclear cells into apoE-knockout mice improved collateral flow in ischemic hindlimbs,76 aortic atherosclerotic lesion size also increased, effects likely explained by the expression of proinflammatory mediators by these cells. However, the complexity of progenitor cell effect on atherogenesis is illustrated by another study71 demonstrating that intravenous injection of a combination of hematopoietic-enriched/stromal-enriched cells obtained from young mice into nonsenier apoE-knockout mice decreased atherosclerotic lesion size. Cells obtained from old mice had no effect. Table 3 lists studies that relate to the possible Janus-like effects of different cell types.10,23,28,53,65

Of interest, the results of a recent, very small clinical trial were compatible with a Janus-like effect of progenitor cells on restenosis. The study was designed to examine the collaterogenic effects of intracoronary infusion of peripheral blood stem cells mobilized with granulocyte-colony stimulating factor in patients with acute myocardial infarction. Although an improvement in left ventricular function occurred that was interpreted as being compatible with a collaterogenic effect, the study was prematurely stopped because of an unexpectedly high incidence of restenosis.76

Direct Relation Between Expansion of Small Arteries and Expansion of Atherosclerotic Plaque

Barger and colleagues77 identified an extensive network of small arteries, originating from the vasa vasorum, as intrinsic to atherosclerotic lesions and possible causally related to increasing lesion size. An expanding vasa vasorum might, for example, supply the atheroma with nutrients and thereby support lesion growth or facilitate entry into the atheroma of inflammatory/immune cells. Additionally, the cells lining these vessels can secrete various cytokines that could cause lesion progression.78 Finally, studies in human carotid endarterectomy specimens79 and in coronary arteries80 suggest that intraplaque microvessels, through microhemorrhages, predispose to macrophage activation and foam cell formation. Although the small arteries constituting the vasa vasorum are not collaterals, it is likely that many of the mechanisms responsible for collateral growth are also operative in the growth of vasa vasorum. If so, then the mechanisms involved in collaterogenesis may contribute to atherogenesis not only by shared signaling and molecular pathways but also by their direct capacity to stimulate increased vascularization of the plaque.

Exception to the Janus Phenomenon

The Janus phenomenon will undoubtedly be found to have notable exceptions. For example, nitric oxide (NO) appears, at least superficially, to be such an exception. Thus, flow-dependent arterial remodeling is endothelium dependent.35 NO plays a fundamental role in the collateral-enhancing effects of vascular endothelial growth factor and of angiopoietin-1,36,37 and endothelial NO synthase (eNOS)–knockout mice exhibit impaired collateral development to hindlimb ischemia.38 Despite these collaterogenic effects, NO exerts antiatherosclerotic activity in vitro81 and in vivo,82,83 suggesting a nonconcordant effect of NO on collaterogenesis and angiogenesis—an exception to the Janus phenomenon.

However, NO is a molecule with complex actions. In addition to synthesizing NO, eNOS catalyzes superoxide formation, which reacts with NO to form peroxynitrite, both of which can oxidize lipoproteins.84 Also, eNOS-deficient mice (with an intact apoE background), when fed a high-cholesterol diet, develop less fatty streak formation than wild-type mice, indicating that in this model NO is proatherogenic.85 In addition, apoE-deficient (apoE-knockout) mice overexpressing eNOS and fed a high-cholesterol diet exhibit greater superoxide production and larger atherosclerotic lesions compared with apoE-deficient mice not overexpressing eNOS.86 Thus, NO does have the potential, under certain experimental circumstances, to increase atherosclerosis, indicating that this molecule is not a clear exception to the Janus phenomenon.

Possible Impact of Temporal Considerations on the Janus Phenomenon

One important consideration, for which there are as yet no experimental data, is the impact of the different temporal patterns on the Janus phenomenon. Thus, atherogenesis develops over months to years, whereas collaterogenesis occurs over the course of days to weeks. It is therefore possible that such temporal differences may blunt the Janus phenomenon, so an intervention designed to enhance collaterogenesis, if active over only a brief time period, may have no substantive impact on atherogenesis.

In summary, we describe a phenomenon—the Janus phenomenon—that posits that when an intervention benefits collateral development, it has the potential to increase atherosclerosis, and when an intervention has antiatherosclerotic effects, it has the potential to inhibit collateral development. Although the general applicability of the Janus phenomenon has yet to be proved, on the basis of mechanistic considerations, its validity has an intuitive resonance. We therefore believe that use of the principles inherent in this phenomenon could be conceptually helpful; any new collateral-enhancing intervention could, by applying the Janus concept, provide mechanistic insights into atherosclerotic mechanisms and visa versa. Most important, the critically important clinical implications of the Janus phenomenon must also be considered when a therapeutic strategy designed to enhance collaterals or prevent atherosclerosis is initiated. In the process of exerting a beneficial effect on the one process, it may be exerting a deleterious effect on the other.

Acknowledgments

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References


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