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 antiatherosclerotic and collaterogenic effects and the actions of which are compatible with the Janus phenomenon.1–18 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, Balb/C mice are resistant to atherosclerosis and have an inferior collaterogenic capacity, 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 propensity 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 Balb/C 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 (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. Compelling data suggest that these same cells and molecules also contribute importantly to collaterogenesis. 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

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Atherogenesis</th>
<th>Collaterogenesis</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>Exacerbates&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Enhances&lt;sup&gt;1–4&lt;/sup&gt;</td>
</tr>
<tr>
<td>FGF-2</td>
<td>Exacerbates&lt;sup&gt;5–6&lt;/sup&gt;</td>
<td>Enhances&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>FGF-1</td>
<td>Exacerbates&lt;sup&gt;6–7&lt;/sup&gt;</td>
<td>Enhances&lt;sup&gt;6,9&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Exacerbates&lt;sup&gt;8,7&lt;/sup&gt;</td>
<td>Enhances&lt;sup&gt;8,9&lt;/sup&gt;</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Inhibits&lt;sup&gt;11–13&lt;/sup&gt;</td>
<td>Inhibits&lt;sup&gt;14&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Exacerbates&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Enhances&lt;sup&gt;15,16&lt;/sup&gt;</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Inhibits&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Inhibits&lt;sup&gt;18&lt;/sup&gt;</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.
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><strong>Adhesion molecules</strong></td>
<td></td>
<td></td>
</tr>
<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><strong>Chemokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (monocyte chemoattractant)</td>
<td>Increases</td>
<td>Increases 10</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><strong>Cytokines</strong></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 41,42</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 22,55</td>
<td>Increases 56</td>
</tr>
<tr>
<td><strong>Metabolic factors</strong></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).

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 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 chemotactic 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 protein simultaneously enhances collaterogenesis in the ischemic hindlimb of apolipoprotein E (apoE)−/− knockout mice and increases atherosclerotic lesion size.

IFN-γ illustrates how the Janus phenomenon can be a hypothesis-generating tool (Table 2). Although evidence indicating an atherogenic effect of IFN-γ is strong, no definitive evidence indicates a collaterogenic effect. The Janus phenomenon suggests such activity. This inference is strengthened in that IFN-γ induces MCP-1 expression, which exerts both proatherogenic and procollaterogenic activity. IFN-γ also induces interferon-γ inducible protein 10 (IP-10) and monokine induced by interferon-γ (MIG) (chemoattractants for T lymphocytes), which are upregulated in regions of collaterogenesis. Moreover, transcriptional profiling of a murine model of atherosclerosis demonstrated upregulation of IP-10 and MIG in response to a proatherosclerotic stimulus, findings compatible with these chemokines playing a role in the initiation and/or progression of atherosclerosis.
intravenous injection of bone marrow–derived mononuclear cells into apoE-knockout mice improved collateral flow in ischemic hindlimbs,70 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 nongenetic apoE-knockout mice decreased atherosclerotic lesion size. Cells obtained from old mice had no effect. Table 3 lists notable exceptions. For example, nitric oxide (NO) appears, although an improvement in left ventricular function occurred that was interpreted as being compatible with a Janus-like effect of progenitor cells. Although the small arteries constituting the vasa vasorum are responsible for collateral growth, it has the potential to increase atherosclerosis, 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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