Granulocyte Colony-Stimulating Factor and Granulocyte Macrophage Colony-Stimulating Factor Exacerbate Atherosclerosis in Apolipoprotein E–Deficient Mice

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Background—Recent studies have suggested a potential contribution of bone marrow–derived progenitor cells to vascular repair. Preliminary clinical studies have explored the possibility that mobilization of progenitor cells with granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) can affect vascular repair. However, it is not known whether the short-term administration of G-CSF or GM-CSF exerts beneficial effects on atherosclerosis.

Methods and Results—Apolipoprotein E–deficient mice were treated with either GM-CSF or G-CSF at a dose of 10 μg · kg⁻¹ · d⁻¹ SC administered daily for 5 days per week on alternating weeks for a total of 20 doses over an 8-week treatment period. We found that in animals maintained on a high-fat diet, both G-CSF and GM-CSF actually demonstrated an increase in atherosclerotic lesion extent. The increase in atherosclerotic extent was not associated with an increase in either inflammatory cells or expression of proinflammatory genes. Interestingly, adventitial vascularity significantly increased, suggesting a mechanistic role for vasa vasorum neovascularization.

Conclusions—These findings demonstrate that in this animal model of atherosclerosis, not only did administration of G-CSF or GM-CSF fail to demonstrate any beneficial therapeutic effect, but both resulted in a worsening of atherosclerosis. (Circulation. 2007;115:2049-2054.)

Key Words: adventitia ■ angiogenesis ■ arteriosclerosis ■ atherosclerosis ■ stem cells

Bone marrow–derived endothelial progenitor cells have been implicated as part of the normal vascular repair mechanism in a number of pathophysiological states in the adult, including atherosclerosis, vascular ischemia, and pulmonary hypertension.1–6 These observations have led to the proposed use of exogenously administered progenitor cells in the treatment of cardiovascular disease.4,5 In animal studies, treatment with bone marrow–derived progenitor cells has been shown to prevent the development of atherosclerosis.7 As a therapeutic alternative to parenterally administering progenitor cells, it has been proposed that mobilization of endogenous, bone marrow–derived progenitor cells with stem cell factors may be a novel approach to enhance cardiovascular repair mechanisms.8

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Animal studies have shown that both granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) can mobilize endothelial progenitor cells.9–11 One recent clinical study demonstrated that GM-CSF can increase angiogenesis and improve cardiac function after myocardial infarction in humans12; another showed no discernible benefit of G-CSF administration.11 Interestingly, to date, no studies have evaluated the potential therapeutic effects of the administration of G-CSF or GM-CSF on atherosclerosis.

On the basis of these observations, we studied the potential beneficial effects of GM-CSF and G-CSF administration on the development of atherosclerosis in the controlled setting of a murine model. We hypothesized that GM-CSF and/or G-CSF would result in a reduction in the extent of atherosclerotic lesion formation.

Methods

Animal Model of Atherosclerosis

Male apolipoprotein E–deficient (ApoE⁻⁻) mice (The Jackson Laboratory, Bar Harbor, Me) 12 weeks of age were placed on either a standard low-fat chow diet (Certified Rodent Chow 5001, Purina, St Louis, Mo) or a high-fat diet (Research Diets, Inc, New Brunswick, NJ). The high-fat diet was formulated to match the original Paigen’s Atherogenic Rodent Diet (30% kcal from fat, 1.25% cholesterol, 0.5% cholic acid). The animals were then divided into 3 groups: a placebo group that received saline injections, a G-CSF group...
group that received murine G-CSF, and a murine GM-CSF-treated group. The dosing for both G-CSF and GM-CSF was 10 μg·kg⁻¹·d⁻¹ SC given daily for 5 days per week on alternating weeks for a total of 20 doses over the 8-week treatment protocol. Systolic blood pressure was measured with a computerized, noninvasive, tail-cuff system (BP 2000 Visitech Systems, Apex, NC) weekly during treatment and before the animals were euthanized. One set of 10 measurements was obtained for each animal, and the mean systolic blood pressure was calculated. Animals were habituated to the device before initiation of treatments to ensure accurate measurements.

After 8 weeks of treatment, the animals were killed by CO₂ inhalation, and blood was collected by cardiac puncture for lipid profiles and measurements of plasma levels of serum amyloid A, tumor necrosis factor-α, and interferon-γ. Plasma lipid analyses were performed by Cardiovascular Specialty Labs (Atlanta, Ga). Triglycerides and total cholesterol were determined by enzymatic methods on a CX5 chemistry analyzer (Beckman Coulter, Fullerton, Calif) with Beckman reagents and controls. Low-density lipoprotein cholesterol was estimated with Beckman reagents and controls. High-density lipoprotein cholesterol was isolated and opened lengthwise. The specimens with hematoxylin and eosin. Macrophages were identified through the homogenous enzymatic kits from Equal Diagnostics (Exton, Pa).

Serum amyloid A, tumor necrosis factor-α, and interferon-γ were assayed with commercially available ELISA kits (Biosource International, Camarillo, Calif, and BD, Franklin Lakes, NJ).

The aortas were pressure perfused in situ with saline and subsequently fixed with formalin. The descending aortas were dissected free for en face analysis of atherosclerotic lesion area using a previously described method. Briefly, the thoracoabdominal aorta from the level of the origin of the proximal descending aorta to the renal arteries was isolated and opened lengthwise. The specimens were pinned open, and digital images of the aortas were acquired for histological analysis, the homogenous enzymatic kits from Equal Diagnostics (Exton, Pa).

In the mice on the low-fat diet, no statistically significant beneficial effect existed from GM-CSF or G-CSF treatment on atherosclerotic lesion area. The mean atherosclerotic lesion areas in the control, G-CSF–treated, and GM-CSF–treated animals were 2.5±0.4%, 3.6±0.9%, and 5.0±1.2%, respectively (n=7 per group; P>0.05). However, in the animals maintained on the high-fat chow, not only was there no evidence of a beneficial effect, but a very significant increase in the atherosclerotic lesion area could actually be seen (Figure 1). Treatment with either G-CSF or GM-CSF more than doubled lesion area in both treatment groups. Although a trend existed for a greater effect with GM-CSF treatment, the difference between the G-CSF and GM-CSF treatments in the high-fat group was not statistically significant. Of interest, 3 animals in the GM-CSF treatment group developed abdominal aortic aneurysms (Figure 1). None of the animals in the G-CSF or control groups developed aneurysms.

Lesion complexity was not altered by treatment with either G-CSF or GM-CSF. The total cross-sectional area of the fibrous cap at the level of the aortic valve was 67 130±6420 μm² in the high-fat group, 64 778±8107 μm² in the high-fat/G-CSF group, and 68 207±5656 μm² in the high-fat/GM-CSF group (n=7 per group; P>0.05). Similarly, smooth muscle cell area was unchanged (40 806±7865, 47 445±8162, and 42 252±1363 μm², respectively; P>0.05).

Potential Mechanisms of Increased Atherosclerosis in G-CSF– and GM-CSF–Treated Animals

Given the very surprising result showing a worsening of atherosclerotic disease extent in the GM-CSF– and G-CSF–treated animals, we attempted to examine the potential mechanisms responsible for this response. Neither G-CSF nor GM-CSF had any effect on blood pressure, as described above. Similarly, serum lipids were not altered by either treatment (the Table).

Both G-CSF and GM-CSF may have either direct or indirect proinflammatory effects. Therefore, we examined the
We could find no significant differences in c-fms expression after 14 days of G-CSF and GM-CSF treatment (Figure 2). Qualitative assessment using immunostaining for macrophages also failed to reveal any significant differences in the treated animals at the same time point (data not shown).

Finally, plasma levels of interferon-γ, serum amyloid A, and tumor necrosis factor-α were measured in animals treated with the atherogenic diet, atherogenic diet plus G-CSF, and atherogenic diet plus GM-CSF. Interferon-γ levels were undetectable at baseline and were not altered by treatment. Serum amyloid A was 2.3±0.7 μg/mL in animals treated with atherogenic diet alone and was unchanged in animals treated with atherogenic diet plus G-CSF (1.8±0.7 μg/mL) or atherogenic diet plus GM-CSF (1.9±0.5 μg/mL). Tumor necrosis factor-α was 31.5±6.2 pg/mL in animals treated with atherogenic diet alone and was unchanged in animals treated with atherogenic diet plus GM-CSF (18.9±5.3 pg/mL). Treatment with the atherogenic diet plus GM-CSF slightly increased plasma levels of tumor necrosis factor-α (55.3±8.6 pg/mL; *P=0.036), an effect that was not consistent with the relative magnitudes of the effects of G-CSF and GM-CSF on atherosclerotic lesion area.

In summary, we found no consistent changes in hemodynamics, local inflammation, lipid profiles, or systemic inflammation that could provide a plausible explanation for the striking effects of G-CSF and GM-CSF treatment on atherosclerotic lesion area.

Differences in Vascularity of G-CSF– and GM-CSF–Treated Animals

Another potentially deleterious effect of G-CSF and GM-CSF is the induction of neovascularization in the arterial wall. As shown in Figure 3, neovascularization within the atherosclerotic lesions and adventitia was markedly increased in the animals treated with GM-CSF (15.3±6.4 vessels per cross section) and G-CSF (18.0±2.5 vessels per cross section) compared with controls (2.0±1.2 vessels per cross section; *P<0.05 versus control).

Discussion

Although stem cell therapy and stem cell mobilization have been heralded as promising treatment strategies for patients with coronary artery disease or peripheral vascular disease, treatment with G-CSF and GM-CSF has not been studied previously in an animal model of atherosclerosis. Prior animal studies focused on acute ischemic situations such as acute limb ischemia or acute myocardial infarction rather than established atherosclerosis.³,10 However, atherosclerosis is present in most clinical situations in which stem cell mobilization is planned. The present study is the first to investigate the effect of short-term administration of G-CSF or GM-CSF on atherosclerotic plaque progression in a murine model of atherosclerosis. More importantly, this study is the first to show not only that administration of G-CSF or GM-CSF fails to exert a therapeutic effect on atherosclerotic lesion extent but also that these agents actually enhance the progression of atherosclerosis. These data raise the possibility that stem cell mobilization with these agents is deleterious.
Despite the relative paucity of data on the effects of G-CSF and GM-CSF on atherosclerosis in animals, clinical trials are being performed on patients with established atherosclerosis. In a nonrandomized study of 16 patients with intractable angina, administration of G-CSF was associated with 2 acute myocardial infarctions and 1 cardiac death. In contrast, G-CSF treatment was not associated with clinical deterioration in any of 10 patients treated with G-CSF in a study of post–myocardial infarction patients who underwent coronary stenting. However, the rate of in-stent restenosis in the G-CSF-treatment group was unexpectedly increased, raising the specter of harmful effects in clinical scenarios in which vessel injury exists. Recently, 2 additional randomized placebo-controlled studies of G-CSF administration in patients after myocardial infarction and successful percutaneous intervention showed neither clinical benefit nor adverse effects. Thus, results from clinical studies are conflicting in terms of both potential benefit and adverse outcomes.

Previous animal studies of the effects of G-CSF and GM-CSF on atherosclerosis are somewhat limited, with 1 previous study demonstrating that the long-term administration of GM-CSF for 7 months in a rabbit model of atherosclerosis had a protective effect. Similarly, a recent publication demonstrated that knocking out GM-CSF in mice exacerbated atherosclerosis. However, these were chronic models that may not recapitulate the type of exposure suggested for mobilizing progenitor cells.

Although G-CSF and GM-CSF have beneficial effects on vascular growth and stimulate collateral blood vessel growth, these agents also have potentially deleterious effects. Our study found that when combined with a high-fat diet, both G-CSF and GM-CSF accelerated the formation of atherosclerotic lesions in ApoE−/− mice as opposed to ameliorating the extent of lesion involvement. In addition, several animals treated with GM-CSF also developed abdominal aortic aneurysms, a finding associated with severe atherosclerosis in ApoE−/− mice. A trend toward a similar effect was seen in the animals treated with a low-fat diet, suggesting that the high-fat diet had a synergistic effect.

One possible mechanism for the enhancement of atherosclerotic plaque area by G-CSF and GM-CSF is the stimulation of an inflammatory state within the arterial wall. This could conceivably occur as a result of mobilization of inflammatory cells. Both G-CSF and GM-CSF stimulate the mobilization and proliferation of monocytes and macrophages. GM-CSF and G-CSF also have direct effects on...
vascular smooth muscle migration and neutrophil activation. These potentially harmful effects may outweigh the putative benefits of these agents. However, because neither inflammatory markers nor macrophage infiltration of the aortic wall was increased during the early stages of atherogenesis, data from the present study do not support a primary role for altered inflammation in the observed increase in atherosclerotic lesion extent.

An alternative potential mechanism for the enhanced atherosclerosis observed in the setting of administration of these stem cell factors is that G-CSF and GM-CSF promote neovascularization in the arterial wall. Indeed, the finding of markedly increased vessel counts in the adventitia of mice treated with the CSFs supports a possible deleterious role for angiogenesis. The association between a more developed vasa vasorum and atherosclerosis was first described in atherosclerotic human coronary arteries. Subsequent animal studies have shown that coronary vasa vasorum neovascularization precedes the development of endothelial dysfunction and atherosclerotic lesion formation. Although the present study clearly demonstrates an association between G-CSF– and GM-CSF–enhanced atherosclerosis and vascularity, causality has not been shown. In previous studies by other groups, the angiogenesis inhibitors endostatin and thrombospondin prevented neointimal formation in the absence of any direct effect on vascular smooth muscle cells. Taken together, these findings strongly suggest a causal role for the vasa vasorum in atherogenesis and provide a potential mechanism for the proatherogenic effects of G-CSF and GM-CSF.

Several technical aspects of this study deserve comment. The doses of G-CSF and GM-CSF used were similar to those used on humans. However, the dosing schedule was more frequent in that the animals received a total of 20 doses over an 8-week period. This is in contrast to 5 to 6 total doses over a 1-week period as used in most clinical trials to date. In addition, although a trend existed for increased atherosclerotic lesion area in animals on a standard chow diet, significant effects of G-CSF and GM-CSF on atherosclerotic lesion area were seen only in animals that also received a high-fat diet. However, clearly no beneficial effect existed from G-CSF or GM-CSF treatment of atherosclerotic disease extent, even in the animals on the standard chow diet.

Conclusions

We have demonstrated an exacerbation of atherosclerosis by G-CSF and GM-CSF treatment. Prior animal models evaluated the effects on acute limb ischemia or acute myocardial infarction simulated by ligation of vessels rather than on atherosclerosis. However, atherosclerosis is almost always present in patients in whom these therapies are being investigated. The mechanism for the deleterious effects of these agents is still not known. Although inflammation is an attractive possibility, this mechanism was not supported by the data at hand. Instead, G-CSF– and GM-CSF–induced atherogenesis was associated with enhanced development of the vasa vasorum. Clearly, further studies are required to delineate causality. In the meantime, the findings presented in this study demonstrate that in this animal model of atherosclerosis, not only did administration of G-CSF or GM-CSF fail to demonstrate any beneficial therapeutic effect, but both resulted in a worsening of atherosclerosis.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Bone marrow–derived endothelial progenitor cells have been implicated as part of the normal repair mechanism in a number of vascular diseases, including atherosclerosis. As a therapeutic alternative to parenterally administering progenitor cells, it has been proposed that mobilization of endothigenous, bone marrow–derived progenitor cells with stem cell factors may provide a novel approach to enhance cardiovascular repair. Therefore, we studied the potential therapeutic effects of granulocyte macrophage colony-stimulating factor stimulating factor on atherosclerosis in the apolipoprotein E–deficient mouse model. We found not only a lack of protective effect of treatment on atherosclerosis with either stem cell factor but also that, in the setting of a high-fat diet, both agents actually increased atherosclerotic lesion extent. These findings suggest that caution should be exercised in the use of granulocyte macrophage colony-stimulating factor or granulocyte colony-stimulating factor as a therapy for atherosclerotic disease. Additional studies are necessary to more fully understand the mechanisms of action of these agents and their potential adverse effects on the vascular wall.
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