VEGF: is it just an inducer of heme oxygenase-1 expression?

Inflammatory reactions are often associated with hemolysis and consequently with the accumulation of free heme released from oxidized hemoglobin. This results in internalization of the free heme by vascular endothelial cells (ECs), where the iron in the core of the heme structure acts as a potent pro-oxidant to promote EC cytotoxicity. 1

The only manner in which ECs can decrease the intracellular levels of free heme is to up-regulate the expression heme oxygenase-1 (HO-1), a stress-responsive enzyme that catalyzes the initial and rate-limiting steps in the oxidative degradation of heme into biliverdin, iron, and carbon monoxide (CO).

We and others have previously shown that the expression of HO-1 prevents the deleterious effects associated with vascular injury, such as the development of arteriosclerotic lesions that arise following EC injury/denudation. 2,3 This protective effect is mimicked by exogenous CO, thus suggesting that it is mediated via the ability of HO-1 to convert heme into CO. 1 An interesting feature in the protective effect of CO is that a single “pulse” of exogenous CO (one hour) seems to be sufficient to block the development of arteriosclerotic lesions, such as those that arise following balloon injury of the carotid artery in rats. 3 This suggests that CO triggers a protective response in cells of the vascular wall that continues to act despite the fact that exposure of these cells to CO has been discontinued. The molecular mechanism underlying this potent and long-lasting protective effect remains to be established.

The article by Bussolati and colleagues that appears in this issue of Blood (page 761) may help to further explain the mechanism underlying the protective effect of HO-1 and CO. The authors demonstrate that vascular endothelial growth factor (VEGF) induces the expression of HO-1 in ECs and, perhaps more important, that HO-1 enzymatic activity in these cells can be critical for the angiogenic effect of VEGF. This adds significantly to the previous observation that HO-1 promotes angiogenesis, 4 in that it directly links the potent angiogenic effect of VEGF to the expression and enzymatic activity of HO-1. In addition, the data obtained by the authors may provide important answers related to the mechanism of action of VEGF in terms of modulating arteriosclerotic lesions triggered by EC injury/denudation. Based on these findings, it is likely that the salutary effects of VEGF are largely due to the expression of HO-1. Induction of HO-1 would not only enhance re-endothelialization of the injured vessels, but it would also protect the newly formed ECs from undergoing apoptosis, suppress the proinflammatory phenotype associated with monocoyte/macrophage (Mø) activation, and inhibit smooth muscle cell proliferation, all of which are key features in the development of arteriosclerotic lesions (reviewed in Soares et al 2).

It is interesting to note that expression of VEGF and HO-1 in ECs may trigger a “positive feedback loop” in which VEGF up-regulates HO-1 and HO-1 up-regulates VEGF. 6 A similar phenomenon has been shown to mediate the anti-inflammatory effect of interleukin-10 (IL-10) in Mø, in which IL-10 up-regulates HO-1 7 and HO-1 up-regulates IL-10. 8 These types of positive feedback loops may explain why a single pulse of exogenous CO, given before vascular injury, is sufficient without further treatment to block the development of arteriosclerotic lesions. 3 This may also explain the potent and long-lasting protective effects of HO-1 and/or CO in a variety of other inflammatory situations.

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Gutted adenoviral vectors in hemophilia A

In this issue, Brown and colleagues (page 804) report results of intravenous infusion of helper-dependent (fully deleted) adenoviral vectors encoding canine factor VIII (FVIII) in dogs with hemophilia A. From these findings and from similar reports from other groups, 1, 2 a picture begins to emerge of the efficacy and toxicity of helper-dependent adenoviral vectors as a gene...
delivery vehicle. Although more data are needed, the findings at this point raise the question of whether these vectors are likely to be useful for the treatment of genetic disease, where long-term expression is the goal.

Adenoviral vectors effect high-efficiency gene transfer into nondividing target cells and remain episomal, thus avoiding the risk of insertional mutagenesis. However, a wealth of preclinical and clinical experience with early generation adenoviral vectors documented short-term expression and immunemediated destruction of transduced cells. Since expression of viral proteins encoded by the vectors was implicated as one of the factors in the destructive immune response, several groups developed fully deleted adenoviral vectors, in which viral coding sequences had been completely removed. Production of such vectors requires a helper plasmid that supplies necessary adenoviral coding sequences in trans, but which cannot be packaged into the recombinant particles. Production and purification methods vary somewhat in the efficiency with which helper virus is removed, a point that may have implications for comparisons among studies from different groups.

An important question in the field has been whether and to what extent the toxicity profile of adenoviral vectors would be modified by the newer generation of fully deleted adenoviral vectors. Toxicities due primarily to innate immune responses to capsid proteins will not be affected by removing viral coding sequences, while those due to expression of viral proteins should disappear. There are 3 recent studies using fully deleted adenoviral vectors in hemophilia A and B dog models that suggest that the toxicity profile is dose-dependent and that adenoviral gene deletion has reduced but not eliminated vector toxicity. Thus, in the current study by Brown et al, a dose of 5 × 10^11 vector particles per kilogram (vp/kg) showed no canine FVIII expression and no toxicity, but a dose of 1.25 × 10^12 vp/kg showed mild transaminase elevation and a drop in platelet count, findings previously documented in large animals with earlier generation adenoviral vectors. A similar study by McCormack et al, using doses of 1 × 10^12 vp/kg and 3 × 10^12 vp/kg in hemophilia A dogs, also showed minor transaminase elevation and thrombocytopenia. On the other hand, a study by Ehrhardt et al in hemophilia B dogs reported no detectable laboratory abnormalities at doses of 6 × 10^11 to 8.5 × 10^11 vp/kg. These data are at least consistent in suggesting a threshold for detectable laboratory abnormalities of approximately 10^12 vp/kg (of fully deleted adenoviral vectors) in dogs. Of course, it is noteworthy that a human subject treated systemically with a dose of 6 × 10^11 vp/kg of an earlier generation adenoviral vector suffered fatal complications, underscoring the importance of defining the source(s) of adenoviral toxicity.

If the toxicities of fully deleted adenoviral vectors in humans are mild and self-limited, as they are in hemophilic dogs, then they would not in themselves argue against further development of the vectors. However, all 3 groups have also described a rapid decline in levels of the transgene product, with only 1 of 8 dogs showing persistent factor levels in a (barely) therapeutic range (dog C in the study of Brown et al, approximately 1% at 5 months after injection). A detailed analysis of the causes of loss of expression will be required to formulate a strategy to avoid this shortcoming. At this point, however, it seems that fully deleted adenoviral vectors, though they exhibit reduced toxicity, still fall short of the goal of sustained expression at therapeutic levels in hemophilia.

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Helicobacter pylori and ITP: many questions, few answers

The factors that incite immune thrombocytopenic purpura (ITP) remain uncertain, though the association of ITP with hepatitis C, cytomegalovirus, and common childhood viruses suggests that infectious agents may contribute. In this issue of Blood, Michel and colleagues (page 890) report their experience investigating the association of Helicobacter pylori (H pylori) with ITP. Of 74 patients, 16 (21.6%) aged 10 years and older (mean age of 41 years) with chronic ITP and a platelet count less than 60 × 10^9/L were infected with H pylori, an incidence not significantly greater than expected for individuals of this age range in developed countries. Although H pylori was eradicated in 14 of 15 treated patients, transient improvement in the platelet count occurred in only 1.

These results contrast with other reports in which a higher incidence of H pylori infection was noted in patients with ITP (Table 5 of Michel et al). Gasbarrini et al documented H pylori infection in 11 of 18 patients; 8 of the 11 patients in whom H pylori was eradicated experienced significant platelet increments.1 Emina et al observed H pylori in 13 of 30 patients with chronic ITP; increased platelet counts occurred in 6 of 12 patients following H pylori eradication.2 However, consistent with the findings of Michel et al, others have reported neither an increased incidence
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