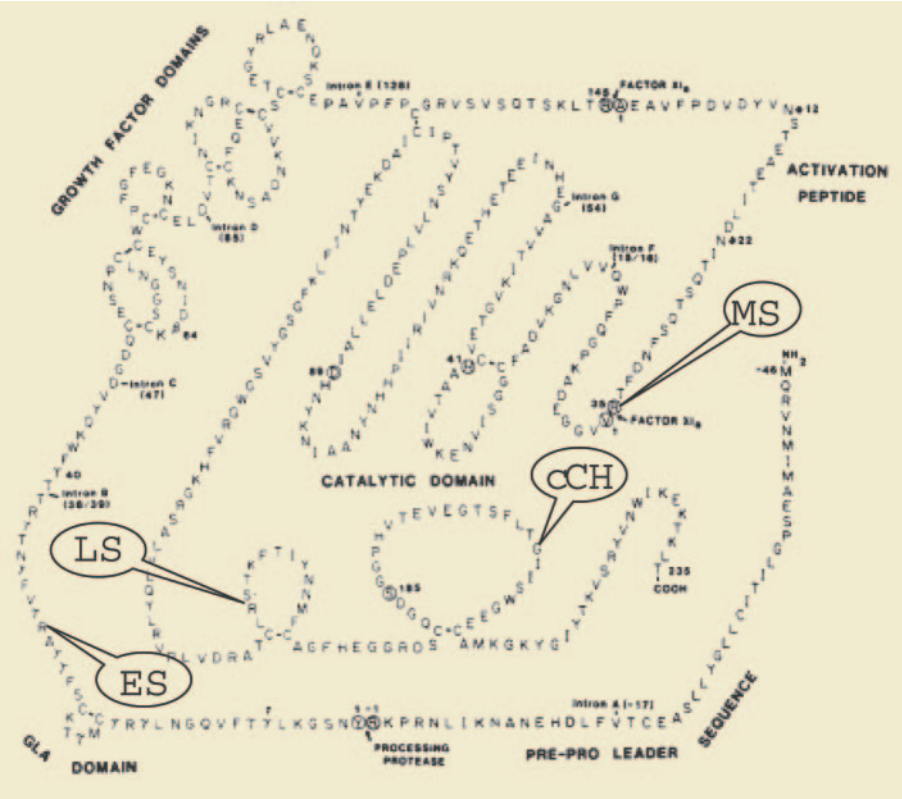


**Human FIX transgenes expressed in mice. See the complete figure in the article beginning on page 2767.**


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HEMOSTASIS

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# The endothelial life insurance plan

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Endothelial-derived apoptotic bodies are ingested by endothelial progenitor cells, resulting in these cells' enhanced proliferation and differentiation. This mechanism may contribute to the repair and maintenance of the vascular endothelium.

The endothelium maintains blood-organ barriers, regulates vasomotor tone, and prevents thrombosis, among other functions. A healthy endothelium is among the most stable tissues, with a turnover time estimated at more than 1000 days. During vascular trauma, angiogenesis, wound healing, and ovulation, however, the turnover rate of the microvascular endothelium may increase dramatically, approaching that of bone marrow cells.[1] If the repair of dysfunctional, eroded, or apoptotic endothelium is inadequate, diseases such atherosclerosis and its ischemic sequelae may ensue.

Despite the clear importance of a healthy endothelium, the mechanisms by which it is repaired are not fully understood. A traditional view has been that arterial injury induces the expression of survival genes and mitogens such as vascular endothelial growth factor that act in a paracine fashion to induce neighboring endothelial cells to proliferate, migrate, and repopulate the damaged surfaces. The discovery of circulating bone marrow–derived endothelial progenitor cells (EPCs)[2] has provided an alternative mechanism in which EPCs contribute to vascular growth and repair in a process that is analogous to vasculogenesis. However, the details by which EPCs are recruited from the bone marrow and home to, proliferate, and differentiate at the specific sites of vascular injury remain obscure.

In this issue, Hristov and colleagues provide intriguing insights into some of these events. EPCs were found to phagocytose apoptotic bodies from endothelial and HL-60 cells. Of most interest was the observation that endothelial-derived (but not HL-60–derived) apoptotic bodies increased the rates of proliferation and differentiation of the EPCs. Additional research is needed to determine the active components of the apoptotic bodies. A reasonable candidate is DNA but other possibilities include sequestered growth factors or membrane-bound receptors. If this effect is confirmed by additional studies, it would represent a significant step forward in EPC biology.

There are several important implications of these findings. Harvested EPCs could be amplified by culturing them in the presence of apoptotic bodies. As the authors note, the uptake of apoptotic bodies by EPCs could be exploited as a novel strategy for molecular therapy. This same phenomenon could also be manipulated to enhance endothelial cell repair or to inhibit angiogenesis in vivo. Patients who have coronary artery disease or risk factors including advanced age[3] and smoking[4] have reduced numbers of circulating EPCs. Dysfunctional adhesion, migration, and proliferation of EPCs have been described in diabetic patients.[5] A failure of EPCs to ingest or to mount a robust proliferative response to endothelial-derived apoptotic bodies could contribute to EPC depletion in conditions that predispose to atherosclerosis. On the flip side, tumor angiogenesis could potentially be abrogated by disrupting these same events.

It appears that the endothelium has a life insurance plan. Understanding the details will produce the maximum long-term benefit for vascular health. ■


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Comment on Ingram et al, page 2752

# Endothelial progenitor cells: reporting for duty

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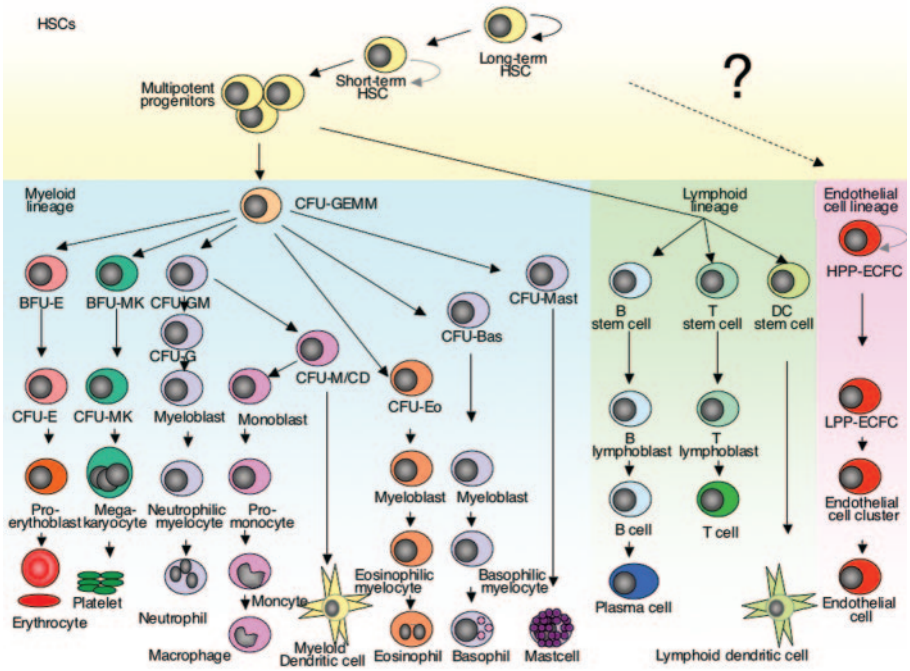
Clonogenic assays define a new hierarchy among endothelial progenitor cells.

The classical view of stem cell differentiation is a hierarchical model that starts with a primitive stem cell at the top, giving rise to a proliferating progenitor pool from which differentiated blood cells develop. As the progeny of stem cells acquire more specific differentiated characteristics they lose their potential to proliferate. The ability for continuous self-renewal is characteristic of the most primitive stem cells. This ability is lost with advancing differentiation. Hematopoiesis is usually seen as a classical example of hierarchical stem cell differentiation and there are many experimental data that support such a hierarchical model.[1-4] A more recent model of stem cell plasticity challenges the classical rigid archetype of hierarchical stem cell differentiation, suggesting that the stem cell, progenitor, and differentiated mature cell relationship is not a fixed hierarchy but rather a reversible continuum (reviewed in Balsam et al[5]).




**Hierarchy of hematopoietic/endothelial stem cell differentiation. The model proposed by Ingram and colleagues implies that endothelial cell differentiation is hierarchical in nature and similar to the differentiation model of the myeloid and lymphoid lineage. The existence of a common progenitor cell that gives rise to both endothelial cells and hematopoietic cells in the adult human bone marrow is of great interest but still remains controversial. The curved arrow indicates self-renewing ability. HSC indicates hematopoietic stem cell; CFU-GEMM, colony-forming unit granulocyte erythrocyte monocyte macrophage; BFU-E, erythroid burst-forming unit; BFU-MK, megakaryocyte burst-forming unit; CFU-GM, granulocyte macrophage colony-forming unit; CFU-G; granulocyte colony-forming unit; CFU-E, erythroid cluster-forming unit; CFU-Eo, eosinophil colony-forming unit; CFU-Baso; basophil colony-forming unit; CFU-Mast; mast cell colony-forming unit; HPP-ECFC, high proliferative potential–endothelial colony-forming cells; LPP-ECFC, low proliferative potential–endothelial colony-forming cells; and EC-cluster, endothelial cell cluster.**

In this issue of *Blood*, Ingram and colleagues use a single-cell colony assay to describe a novel hierarchy amongst human endothelial progenitor cells (EPCs) isolated from peripheral blood and umbilical cord. The identification of a distinct population of progenitor cells is based on their clonogenic and proliferative potential. High-proliferative potential–endothelial colony-forming cells (HPP-ECFCs) are large colonies that form secondary and tertiary colonies upon replating. HPP-ECFCs give rise to all subsequent stages of EPCs to secondary HPP-ECFCs. Low-proliferative potential–endothelial colony-forming cells (LPP-ECFCs) form colonies of more than 50 cells but no secondary LPP-ECFCs upon relating. Endothelial cell clusters (EC clusters) contain fewer than 50 cells and show recognizable features of differentiation; finally, the mature endothelial cells do not divide (Figures 1 and 7 in the paper by Ingram and colleagues in this issue of *Blood*). There has been a multitude of prior studies that describe the isolation of EPCs from human peripheral blood or umbilical cord. However, many of these so-called EPCs, although expressing some of the markers shared by both hematopoietic cells and EPCs, contained $CD14^+$ cells that maintain the expression of the hematopoietic surface antigen CD45. Although these cells seem to support angiogenesis in vivo, this seemed to occur through the secretion of factors promoting angiogenesis rather than through proliferation and contribution to the lining of the neovasculature. Thus, these cells seemed more like circulating angiogenic cells than EPCs.[6] The endothelial cells that outgrow from the progenitor cells isolated by the method described by Ingram and colleagues showed phenotypic and functional characteristics of endothelial cells and lack hematopoietic characteristics such as CD45 and CD14. Genetic studies in mice