more likely in older patients, but they achieved remission faster with immunosuppressive therapy. Underlying diagnoses, bleeding complications, and response to hemostatic therapy confirmed previous reports; however, bleeding symptoms were more varied, with 33% requiring no hemostatic therapy and 8% with fatal bleeding.

Collins and colleagues then analyzed the outcome of 2 basic immunosuppressive treatment regimens administered to 88 of the patients: steroids alone (n = 40; usually given as prednisolone, 1 mg/kg per day) versus steroids combined with cytotoxic therapy (n = 48; usually cyclophosphamide, 1-2 mg/kg per day). They found no differences in the proportion of patients who achieved complete remission (CR) or in the time to CR; furthermore, addition of other therapy such as intravenous immunoglobulin (IVIG) did not alter the results. The one caveat to this observation is that this was not a randomized study. Physicians were allowed to choose the immunosuppressive regimen; this led to treatment with steroids alone in patients with a lower median inhibitor titer (8 BU) than those treated with combination therapy (18 BU); P < .01. Thus, it is possible that the outcomes are biased by more intense treatment of the more severely affected group. Nevertheless, this study provides us with the best data to date from a large group of patients. Only one randomized clinical trial has been reported, a comparison of the addition of cyclophosphamide to steroid treatment versus continuation of steroids after 3 weeks; there were no differences in outcome, but the study was quite small.3

Collins and colleagues are to be congratulated for their major effort to identify all patients presenting with acquired hemophilia A. They have provided us with the most unbiased description of the disorder to date; in this case, more data is clearly better. The question of whether more treatment is better, either more standard therapy as described here or use of new agents such as rituximab, awaits additional well-designed clinical trials.

The author does not competiting financial interests.

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Comment on David et al, page 1953

Bone morphogenetic proteins go endothelial

Danny Huylebroeck  FLANDERS INSTITUTE FOR BIOTECHNOLOGY, DEPARTMENT OF DEVELOPMENTAL BIOLOGY, KU LEUVEN

Mouse knock-out studies, joined later by studies in zebrafish, have shown that the transforming growth factor type β (TGFβ) receptors are essential for angiogenesis, while mutations in the endothelial cell–specific and signaling receptor for TGFβ, ALK1, are associated with the genetic vascular disease hereditary hemorrhagic telangiectasia (HHT) type 2. Now, bone morphogenetic proteins (BMPs) join this stage.

Studies in cell culture have suggested that angiogenesis is regulated by a switch between 2 TGFβ signaling pathways, one emanating from (the ubiquitously expressed) ALK5, the other one from ALK1.1,2 This switch is regulated by the coreceptor endoglin present on angiogenic vessels, and endoglin mutations cause hereditary hemorrhagic telangiectasia (HHT) type 1. ALK5 activates in cultured endothelial cells the downstream Smad proteins Smad2/3, whereas ALK1 acts through Smad1/5/8. Smad1/5/8 are bone morphogenetic protein (BMP) Smads because they are activated by the BMP receptors ALK2, ALK3, and ALK6 in tissues ranging from cartilage and bone tissue to many soft tissues in the embryo and the adult animal. Candidate target genes for each of the 2 TGFβ pathways in mainly human umbilical vascular endothelial cells (HUVECs) but also human microvascular endothelial cells (HMECs) have been identified.3-5 For this, either stimulation of the cells with TGFβ was used or overexpression of a constitutively active form of ALK1 and ALK5.

In this issue of Blood, David and colleagues report important new results on the signaling activity of ALK1 in HMECs from dermis (HMEC-d’s). The authors were intrigued by recent results on the crystal structure of BMP9 (also named GDF2) demonstrating that BMP9 can bind to receptor complexes consisting of ALK1 and BMP type II receptor (BMPRII) and that complex and mature forms of BMP9 exhibit similar biologic activity.6 Some groups have reported, mainly at meetings, preliminary data on BMP binding (in particular BMP2, BMP4, and BMP7 have been tried as these were initially the only ligands widely available) to ALK1. For the many BMP ligands with possible therapeutic potential, the affinities of the individual ligands for the multiple combinations of BMP receptors are well-kept secrets. To date, no convincing data have emerged showing that a BMP is a physiologic ligand for ALK1 in the vessel wall. David and colleagues show that BMP9 (and BMP10) induces in HMEC-d’s sustained Smad1/5/8 phosphorylation and activates transcription of a BMP-Smad reporter gene and the BMP-Smad target gene Id1. Using siRNA approaches and others, the authors show that this stimulation is dependent on ALK1 (and BMPRII), while cotransfected endoglin increases the response to BMP9. David et al also demonstrate that BMP9 (and BMP10) induces a number of genes that have been identified in each of the microarray studies done so far and that searched for ALK1 target genes.3-5

The results of the paper by David and colleagues add endothelial cells as targets for certain ligands of the BMP subgroup of the TGFβ family. This work will attract the attention of many groups studying the in vivo function of BMP signaling in various processes in embryogenesis and disease. Future challenges are to include the documentation of Smad but also non-Smad signaling emanating from ALK1–TβRII and ALK1–BMPRII complexes when activated by TGFβ and BMP9, respectively, and where in vivo the
BMP9→ALK1 pathway is operational and relevant. Also, the role of the coreceptor endothelin will have to be revisited and perhaps other coreceptors should be searched for. Alternative approaches would be those that involve the identification of proteins that interact with the intracytoplasmic tail of TβRII and/or BMPRII. Such proteins may lead to a better understanding of how the signaling and perhaps the endocytic routing of these ligated receptors, and thereby the cellular response, is regulated, or whether in the case of BMP ligands preformed versus BMP-induced receptor complexes are assembled and kept separated, including in endothelial cells.

The author declares no competing financial interests.

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Comment on Yoder et al, page 1801

Will the real EPC please stand up?

Aernout Luttun and Catherine M. Verfaillie

In this issue of Blood, Yoder and colleagues take a step forward in resolving the existing controversy on the origin and functional definition of endothelial progenitor cells (EPCs). The authors demonstrate that the mononuclear cell fraction of peripheral blood harbors 2 clonally distinct progenitor populations, one with true endothelial differentiation potential in vitro and in vivo and another myeloid precursor, lacking this potential.

Since their initial description almost 10 years ago,1 endothelial progenitor cells (EPCs) have met with great enthusiasm given the therapeutic promise they hold, as well as much confusion and controversy evidenced by the growing body of literature discussing their origin, molecular signature, and endothelial differentiation capacity in vitro and in vivo.2 The prospect of being able to supply/recruit functional endothelial precursors in ischemic patients as an alternative for/in addition to existing surgical or angiogenic growth factor treatments for revascularization has prompted many researchers to engage in testing the therapeutic potential of these cells in many animal species and patients. However, contradictory findings have been reported concerning the nature of EPCs and their actual contribution to blood vascular endothelium, not only in an ischemic context but also in other processes involving neovascularization, such as tumor growth. Several researchers have highlighted that EPCs phenotypically resemble monocytes and may even be derived from them.3,4 Many early studies have claimed a significant—up to 50%—direct contribution of EPCs to newly formed vessels, while more recent studies have demonstrated that vascular incorporation of EPCs (and bone marrow—derived cells in general) is minimal and have suggested that their vascular potential may be mainly—if not exclusively—trophic, in that they supply angiogenic growth factors to host vascular cells.5

The controversy probably finds its origin in a combination of factors, some of which are related to variations in the research protocol and others that may be out of control of investigators. As an example of the latter, given the formidable heterogeneity of the endothelium throughout the body, it is likely that direct vascular contribution may not be as efficient in every vascular bed or that not every organ is as accessible/permisive for EPCs recruited from the peripheral blood—hence the variable direct contribution of EPCs. However, it is becoming clear that a large part of the variability comes from the differences in cell populations used in different studies because of perhaps small but biologically significant differences in the methodology to derive and culture them. Here, Yoder and colleagues clearly demonstrate that, starting from the same source (namely, peripheral blood mononuclear cells), 2 fundamentally different cell populations can be generated by varying the culture conditions. The simple variable that determined the nature of the resulting cell population was whether adherent or nonadherent cells were propagated in culture. When the adherent fraction was kept, colonies grew out late, which the authors call endothelial colony-forming cells, or ECFCs. Such ECFCs have not only the molecular but also functional characteristics of endothelial cells (ECs) in vitro and in vivo. If the nonadherent cells were replated and kept in culture, cell colonies emerged earlier with a mixed molecular signature that includes EC markers, but with functional characteristics of monocyte/macrophages, rather than ECs. These cells are referred to as endothelial cell colony-forming units, or CFU-ECs.

This is not the first study reporting the similarities of EPCs with monocytes.3,4 However, in the current study the authors systematically “dissect out” the origin and in vitro and in vivo functional characteristics of these “monocyte-like EPCs” (CFU-ECs) as well as their true endothelial counterparts (ECFCs). Using clonal studies, the authors definitively prove that CFU-ECs are clonally related to hematopoietic stem cells, but not to ECFCs. CFU-ECs not only have monocyte/macrophage surface marker expression but also have myeloid colony-forming capacity and give rise to functional macrophages. Of most importance, unlike ECFCs, CFU-ECs were not able to incorporate into vessels in vivo, which may well explain why some previous reports have failed to find vascular integration of presumed EPCs following transplantation.

In summary, this study provides functional criteria for the definition of EPCs, or as termed by the investigators, ECFCs, and resolves some of the ongoing confusion in the field of postnatal vasculogenesis. The most important
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Danny Huylebroeck