BMP9→ALK1 pathway is operational and relevant. Also, the role of the coreceptor endoglin 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 liganded 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.

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hemostasis

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Will the real EPC please stand up?

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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
feature, after all, for a cell to be called an EPC is its ability to differentiate into an EC and directly participate in vessel growth. While ECFCs comply by these rules in a matrix implantation model, additional studies will be needed to determine if ECFCs behave similarly in an ischemic environment and contribute to new vessel formation. By all means, the authors’ note that it is time to clearly define and characterize cell populations does not apply to EPCs only, but is an important take-home message for everyone involved in (stem) cell research.

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Comment on LeMaoult et al, page 2040

**Fast track to becoming a regulatory T cell: “trogocytosis” of immune-tolerogenic HLA-G**

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LeMaoult and colleagues have identified a challenging novel mechanism that effector T cells can use to immediately acquire regulatory function. The mechanism relies on the direct and rapid exchange of membrane fragments between APC and T cells, a process called trogocytosis. Uptake of immune-tolerogenic HLA-G by some resting but most activated CD4 and CD8 T cells leads to the instant generation of a new type of regulatory cells that initially act through cell–surface molecules that they temporarily display but do not express themselves.

Cell-to-cell contact-dependent transfer of membrane fragments and associated molecules from one cell to another was first noticed in the early 1980s. This phenomenon has recently been named trogocytosis and meanwhile described in a number of cells in mice and humans, in most cases immune cells at the antigen–presenting cell (APC)–T-cell interface.

The hallmarks of trogocytosis are the requirement of cell-to-cell contact and possibly immune synapse formation. Transfer via trogocytosis is fast, efficient, requires no more than a few minutes, and includes cell-surface, intramembrane, adaptor, and attached intracytoplasmic molecules (nonselective process). Various cell subsets can interact with each other (APCs–T cells, target cells–natural killer [NK] cells, peripheral cells–APCs); in most instances a predominant ligand–receptor interaction facilitates the exchange of material (MHC–TCR, CD28–B7, CD54–LFA, MHC–I–KIR). The specific physical environment seems to dictate the process, which can be antigen–specific. Most of the work known to date concerns APC–to–T-cell transfer in the murine system: CD4 and CD8 T cells acquired APC MHC class II and MHC class I molecules in an antigen–specific manner. TCR engagement seems necessary for trogocytosis to occur in most systems, since T-cell activation or anti-CD3 treatment increases efficiency of membrane exchange. In some systems, trogocytosis depends on CD28 engagement, and it was shown that costimulatory molecules (eg, B7–1 or ICAM–1) are acquired by murine antigen–specific T cells.

The detailed mechanisms by which these transfers occur, and even more so their overall functional and physiological significance, have remained largely elusive. Three principal possibilities are being considered (see figure): (1) immune effector clearance: acquisition of cognate MHC class I ligands by CD8 T cells predisposes cytotoxic T lymphocytes (CTLs) to “fratricide” antigen-specific cytolyis, thereby contributing to the clearance of CD8 effector cells; (2) amplification of immune responses: trogocytic transfer of stimulatory membrane portions to CD4 T cells (especially MHC class II and B7-costimulatory molecules, also called “presentasomes”) might constitute an efficient way to increase the immune system’s antigen–presentation and stimulation capabilities by generating APC-like T cells; and (3) sustenance of immune responses in the absence of APCs: MHC class I and CD80 acquired from APCs regulate T-cell proliferation and sustain their activation in the absence of APCs.

Critics of this concept allege that trogocytosis—albeit possibly playing a role from a quantitative standpoint—should have no major physiological relevance, since it would not induce qualitative changes of immune responses under in vivo conditions (ie, changing the repertoire of immune cells).

Starting from this point, LeMaoult and colleagues reasoned that trogocytosis might be useful if “unusual” molecules limited in their expression in time and space and/or equipped with unusual function came into play instead of MHC molecules expressed by every APC. HLA-G, a nonclassical MHC molecule characterized by strong immunosuppressive function and highly restricted tissue expression under physiological conditions, was chosen as a paradigm. HLA-G plays a key role in mediating immune tolerance at the maternal–fetal interface. Ectopic or neoexpression has been described in cancers, transplantation, inflammatory/autoimmune disorders, and some viral infections, where HLA-G contributes to immunopathology via its overall negative immune–regulatory functions (such as inhibiting allogeneic proliferation of T cells, NK-cell cytotoxicity, and antigen-specific cytotoxicity).3

In an elegant series of in vitro experiments, the authors show that (1) polyclonal CD4 and CD8 T cells acquire HLA-G from APCs (HLA-G1–expressing LCL–721.221, IFN–gamma–stimulated monocytes) by membrane exchange and (2) that acquisition of HLA-G through membrane transfer immediately reverses their function from effectors to regulatory cells. HLA-G1 was transferred along with HLA-DR, CD86, CD54, and ILT-2 (but not ILT-3) and...
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