Comment on Min et al, page 1495

Novel function of RANKL: eNOS activator

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In this issue of Blood, Min and colleagues report that RANKL is a potent activator of endothelial nitric oxide synthase (eNOS) and induces vascular hyperpermeability and angiogenesis in an eNOS–NO– dependent manner.

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teonceptor activator of nuclear factor (NF)–κB ligand (RANKL, also known as ODF, osteoclast differentiation factor; OPGL, osteoprotegerin ligand; and TRANCE, TNF-related activation-induced cytokine) is a key regulator of bone homeostasis, lymphocyte development, lymph node organogenesis, and mammary gland formation.1 RANK is a signaling receptor of RANKL—which is counterbalanced by a decoy receptor OPG. In addition to the well-studied effects on the skeletal and immune systems, the OPG/RANKL/RANK system appears to mediate vascular pathology such as atherosclerosis, calcification, and plaque destabilization. However, its physiologic role and downstream signaling pathways in the vascular system are not known.

Nitric oxide (NO) is a multifunctional gaseous molecule that plays important roles in blood vessel physiology and pathology.2 NO mediates endothelial-cell migration and proliferation and recruitment of endothelial precursor and perivascular cells, and thus, facilitates angiogenesis and vessel maturation. NO regulates vessel tone and blood flow via relaxation of vascular smooth muscle cells. NO also inhibits platelet aggregation and leukocyte adhesion, and mediates vascular hyperpermeability in pathologic settings. Endothelial NO synthase (eNOS) predominantly mediates these vascular functions of NO.

Kwon and colleagues previously reported that RANKL activates vascular endothelial cells and induces adhesion molecule expression, endothelial tube formation, and angiogenesis in vivo (Kim et al3 and Min et al4). Now Min and colleagues show that for the first time RANKL increases vascular permeability and that various RANKL-induced vascular events—leukocyte extravasation, increased permeability, and angiogenesis—are mediated by eNOS (see figure). Exogenously applied RANKL phosphorylates Ser1177 in eNOS via the tumor necrosis factor receptor–associated factor 6 (TRAF6) and PI3K–Akt pathway. eNOS–NO mediates RANKL–induced endothelial-cell migration and tube formation as well as angiogenesis in a Matrigel assay. NO also induces VE-cadherin relocation from endothelial-cell membrane to cytosol and increases leakage of Evans blue dye in the skin and FITC-dextran in the retina. These findings shed new light on the role of RANKL in the vascular system, and reveal the central role of eNOS as a mediator of RANKL’s function in the vasculature.

This exciting study also raises many unanswered questions. For example, what is the role of endogenous RANKL in the vasculature under physiologic or pathologic conditions? What are the spatial and temporal regulations of endogenous RANKL/OPG/RANK during angiogenesis and/or inflammation? Can the causal role of endogenous RANKL on eNOS and vasculature be unraveled by blocking its signaling with OPG, OPG-like proteins, soluble RANK, or RANKL neutralizing antibodies?

It is noteworthy that the effects of RANKL are not exclusively eNOS–NO dependent. For example, a NOS inhibitor or eNOS deletion significantly diminishes, but does not completely abolish, the effects of RANKL. Similarly, dominant-negative TRAF2 expression...
or a Src inhibitor blocks RANKL-induced endothelial-cell migration but does not affect eNOS activity. As originally discovered, RANKL activates NF-κB via activation of IKKα. In turn, NF-κB mediates up-regulation of cell-adhesion molecules such as ICAM-1 and VCAM-1 induced by RANKL. 4 NF-κB is also a potent inducer of inducible NOS (iNOS). iNOS may be involved in and/or interfere with RANKL functions in vivo. Thus, NF-κB may also affect RANKL-induced angiogenesis and vascular hyperpermeability. Finally, the reduction in RANKL-induced leukocyte extravasation in eNOS−/− mice is counterintuitive. NO is known to down-regulate cell-adhesion molecules including ICAM-1 and VCAM-1. One would expect to see increased leukocyte adhesion in eNOS−/− mice rather than a decrease. Further studies to resolve this apparent discrepancy are needed. In the meantime, the report by Min and colleagues opens new connections between the fields of vascular biology and inflammation.

The authors declare no competing financial interests.

REFERENCES


HEMATOPOIESIS

Comment on Tober et al, page 1433, and Ghinassi et al, page 1460

On the origins of megakaryocytes

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Megakaryocytes and erythroid cells share a common precursor, the megakaryocyte/erythroid progenitor (MEP). Two reports in this issue of Blood provide new insights into megakaryocyte ontogeny and the plasticity of MEPs.

Shortly after gastrulation, fetuses must produce their own supply of red blood cells to transport nutrients and nourish the embryo. This is accomplished in 2 stages: primitive erythropoiesis, which initiates in the blood islands of the yolk sac, and definitive erythropoiesis, which predominantly occurs in intraembryonic sites such as the fetal liver.

Although multiple studies have shown that erythroid cells share a common precursor with megakaryocytes during definitive hemopoiesis and that megakaryocytes can be generated from yolk sac tissue,1,2 the relationship between these 2 cell types during primitive blood development has remained unclear. Furthermore, the developmental stages during which embryonic platelets are produced, and the requirements for platelets in utero, have not been well defined.

To investigate the ontogeny of megakaryocytes in mice, Tober and colleagues evaluated megakaryopoiesis in murine embryos beginning at E7.0. Using a combination of progenitor assays and immunohistochemistry, they discovered that the capacity to produce megakaryocytes coincides temporally and spatially with primitive and definitive waves of erythropoiesis. In addition, consistent with the existence of a bipotential primitive megakaryocyte/erythroid progenitor (MEP), they identified single colonies containing both primitive erythroid (βH1-globin positive) and megakaryocytic (GPIbβ-positive) cells (see figure). Serial dilution assays suggest that, similar to definitive hemopoiesis, a single progenitor cell can give rise to both lineages during primitive hemopoiesis. Do these progenitors give rise to platelets in vivo? To answer this question, Tober and colleagues assayed for megakaryocytes and platelets in both the yolk sac and the embryo proper. Rare GPIbβ-positive cells corresponding to megakaryocytes were detected in the yolk sac at E9.5 and within the fetal liver by E10.5. Surprisingly, platelets were first detected as early as E10.5 in peripheral blood pooled from multiple embryos, and subsequently in all fetuses by E11.5. These early platelets are larger than those circulating within late-stage fetuses and adults, and contain smaller α-granules and a larger open canalicular system. Also, in contrast to adult platelets, the vast majority of E11.5 platelets was reticulated. Although these differences may reflect the maturation stage of the megakaryocytes from which the platelets were derived, it is tempting to speculate that primitive platelets are morphologically distinct from their more mature counterparts in the same way that primitive erythroid cells are larger than definitive erythroid cells.

MEPs are defined by their limited potential to give rise to erythroid and megakaryocytic cells. Both of these lineages require the transcription factor GATA-1 for their terminal maturation, but the function of GATA-1 in the MEP has not been extensively studied. Mice engineered to express reduced levels of GATA-1 (GATA-1−/+ mice) display thrombocytopenia throughout their lifetime and eventually develop anemia with myelofibrosis and extramedullary hemopoiesis.3 In addition, these mutants show defects in mast-cell development and an accumulation of unique tri-lineage cells committed to the erythroid, megakaryocytic, and mast-cell lineages.4 In this issue of Blood, Ghinassi and colleagues demonstrate that single immunophenotypic MEPs isolated from GATA-1−/+ mice, which express 4-fold less GATA-1 than wild-type MEPs, have the remarkable ability to generate not only red blood cells and megakaryocytes, but also mast cells. These latter cells are normally derived from mast-cell precursors that branch off from the common myeloid progenitor (CMP) opposite to and distinct from the MEP. These observations demonstrate that...