the adaptor protein DAP12, which triggers downstream signaling. TREM-1 expression is up-regulated by lipopolysaccharide and other microbial products and it functions as an amplifier of the inflammatory response in the context of microbial infections. Bosco et al. show that HIF-1 is, at least in part, implicated in the hypoxic-mediated transcriptional activation of TREM-1, consistent with the presence of a putative hypoxia response element in the promoter region. Notably, cross-linking of TREM-1 was associated with induction of DAP12-mediated signaling pathways leading to activation of AKT, ERK-1, and IκBα and increased production of pro-inflammatory cytokines, indicating that TREM-1 may be involved in amplifying hypoxic-dependent inflammatory responses. TREM-1 ligand is still unknown, so it is unclear how hypoxic conditions trigger TREM-1/DAP12 signaling cascade. Notably, TREM-1 signaling was primarily activated under chronic, rather than acute, hypoxic conditions, emphasizing the requirement for sustained stimulation to activate TREM-1 expression and further indicating a functional distinction between normoxic and H-mDCs.

The findings reported by Bosco et al. in this issue raise a number of questions. What are the functional consequences of TREM-1 expression in H-mDCs? Does TREM-1 expression affect the response of mDCs to hypoxia? In which other cell types is TREM-1 induced by hypoxia? More importantly, what is the role of TREM-1 in human pathologic conditions known to be associated with hypoxia? Bosco et al. provide evidence that this pathway may be activated in synovial fluid from children with Juvenile Idiopathic Arthritis, an inflammatory condition associated with hypoxia. Previously, TREM-1 expression had been implicated in the pathogenesis of sterile inflammatory conditions, including rheumatoid arthritis. Further studies are required to fully appreciate the role of TREM-1 expression in human inflammatory diseases associated with hypoxia, either as a marker of H-mDCs or as a potential therapeutic target.

Overall, this study highlights several important points. The first is the essential contribution of tissue hypoxia to the initiation and amplification of inflammatory processes, emphasizing its potential involvement in a broad range of human diseases. The second is the heterogeneous response of distinct cellular components to the hypoxic inflammatory mi-
croenvironment, which may differentially modulate the inflammatory response. Finally, the recurrent implication of hypoxia-triggered inflammation in distinct, and apparently unrelated, human diseases provides an opportunity to identify novel targets and develop more effective therapies potentially active in a broad range of pathologic conditions.

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Comment on England et al, page 2708

Self-renewal in late-stage erythropoiesis

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Extensive in vitro self-renewal of proerythroblasts from the earliest period of definitive erythropoiesis, as reported by England and co-investigators in this issue of Blood, expands the potential role of self-renewal in mammalian hematopoiesis and suggests a possible source of blood cells for clinical treatments.

In standard hematopoietic hierarchy models, the pluripotent hematopoietic stem cell (HSC) undergoes long-term self-renewal cell divisions that create more HSCs. The HSC also commits to differentiation by cell divisions that form all lineages of lymphohematopoietic cells. In diagrams of these models, HSC self-renewal is designated by a reflexive arrow, while another arrow leads to a sequence of bifurcating stages of hematopoietic differentiation that terminates in the mature cells of the blood and lymphoid tissues. The proliferative potential of progenitor cells at each successive stage of differentiation is generally assumed to be less than in the previous stage. Other than the HSC, none of the subsequent progenitors in these differentiation schemes was believed to have the potential for long-term self-renewal. Now, England et al report that, soon after the genesis of definitive erythropoiesis, the relatively late differentiation stage of the proerythroblast has the capacity for extensive self-renewal divisions in vitro. In the figure, this capacity for extensive self-renewal is represented by a reflexive arrow not only at the HSC stage but again at the proerythroblast stage.

The sequence of differentiation stages for the erythroid lineage includes the HSC, the common myeloid progenitor (CMP), the bipotential megakaryocytic-erythroid progenitor (MEP), and then a sequence of erythroid-restricted progenitors and morphologically distinguishable precursors, as shown below the dashed line in the figure. The erythroid-restricted progenitors were originally defined...
by their growth into colonies in semi-solid tissue culture, with the earlier burst-forming units—erythroid (BFU-E) having much greater proliferation potential than the subsequent colony-forming units—erythroid (CFU-E). Proerythroblasts bridge the differentiation gap between the functionally defined CFU-E and the morphologically defined precursor stages of basophilic through orthochromatic erythroblasts. However, proerythroblasts have a relatively nonspecific morphologic appearance, and they likely overlap functionally with mature CFU-E. In addition, CFU-E and proerythroblasts are the last stages in the erythroid differentiation sequence with kit ligand/stem cell factor (SCF) responsiveness and the earliest stages with erythropoietin (EPO) dependence.

Most previous studies of committed erythroid progenitors were performed with tissue culture media that contained EPO with or without SCF, and they confirmed the progressive loss of proliferation potential from immature BFU-E that could produce tens of thousands of terminal cells to CFU-E that divided only 3 to 6 times before reaching the terminal nonproliferative stage of orthochromatic erythroblasts. However, addition of the synthetic glucocorticoid hormone dexamethasone greatly expanded the in vitro proliferation of human erythroid progenitors that were also dependent on EPO and SCF in the culture medium. These expanded erythroid cells arose from enriched populations of BFU-E, CFU-E, and proerythroblasts, and they remained undifferentiated until they were cultured in medium without SCF and dexamethasone. When cultured without these 2 hormones, the expanded cells differentiated to orthochromatic erythroblasts and reticulocytes (EB and Retic in figure) within 4 to 5 days. Thus, EPO, SCF, and dexamethasone were required for a very large, but ultimately limited, in vitro expansion of the erythroid precursors because of cell divisions that led to self-renewal rather than differentiation. Mice with mutant glucocorticoid receptors had greatly impaired expansions of CFU-Es in response to hemolysis and hypoxia, indicating that stress erythropoiesis in vivo required glucocorticoids as well as SCF and EPO.

Using the same 3 growth factors, England et al now demonstrate that proerythroblasts formed at the very beginning of definitive erythropoiesis in mid-gestation fetal mice can sustain in vitro self-renewal divisions much longer than proerythroblasts from older fetuses or adults. When they are induced to differentiate by culture in medium without dexamethasone, the proerythroblasts require only 3 to 4 cell divisions to reach the terminal erythroid precursor stage, the reticulocyte. During these terminal cell divisions, proerythroblasts undergo the major biochemical and morphologic events of terminal mammalian erythroid differentiation such as hemoglobin accumulation, reduced cellular size, and enucleation. This terminal erythroid differentiation from the proerythroblast to the reticulocyte requires approximately 2 days in mice and 4 to 5 days in humans. Thus, extensive self-renewal of proerythroblasts permits the accumulation of a very large number of cells that have not entered into terminal erythroid differentiation, but they can be induced by changes in growth factor concentrations to complete differentiation into RBCs within days.

Whether extensive self-renewal of proerythroblasts has a role in normal fetal and early postnatal life is unknown. However, body size and blood volume increase very rapidly during the perinatal period of murine development, and self-renewal of proerythroblasts may help meet the demand for increased...
red cell production. The extensive self-renewal of these relatively late-stage erythroid cells in vitro raises the question of whether the earlier stages of erythroid differentiation or corresponding later stages in other hematopoietic lineages may harbor similar extensive capacities to self-renew. If they do, then understanding the cellular mechanisms that permit extensive self-renewal divisions of various progenitor stages might provide sources of nonerythroid as well as erythroid blood cells for clinical use.

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Breakup feared after filamin leaves GPIb
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In this issue of Blood, Cranmer and colleagues demonstrate that the linkage between the cytoskeletal protein filamin A and the platelet receptor glycoprotein (GP) Ibα provides structural integrity to the plasma membrane during platelet adhesion to von Willebrand factor (VWF) under high shear.1

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On damage to vessel walls, exposed extracellular matrix (ECM) proteins trigger a series of events leading to the formation of a vascular plug. ECM–associated VWF plays a critical role in mediating initial platelet recruitment under flow because of the rapid on-rate of binding between VWF and the platelet receptor GPIb/V/IX.2 A conformational change in VWF induced by binding to ECM proteins is required for GPIb–VWF interactions, restricting platelet recruitment to sites of vascular injury. Alternatively, high-affinity binding of GPIb to VWF can be induced by exogenous modulators such as the bacterial glycopeptide ristocetin, the snake C-type lectin botrocetin, or pathologic levels of high shear as is found in stenosed vessels.3,4

Concomitant with mediating platelet recruitment to VWF, the GPIb/V/IX receptor complex regulates the cytoskeletal architecture of platelets. The GPIb/V/IX complex is physically anchored to the membrane skeleton through the cytoskeletal protein filamin A, and is intimately involved in maintaining platelet size and shape.4,5 Disrupted expression or mutations in the GPIb/V/IX complex in Bernard-Soulier syndrome results in a bleeding phenotype characterized by giant platelets and thrombocytopenia.6 Similarly, mice with filamin-deficient platelets have macrothrombocytopenia, increased bleeding times, and decreased expression and altered surface distribution of GPIbα.7

Filamin A is composed of 2 identical subunits, each containing an N-terminal actin-binding domain followed by 24 tandem immunoglobulin-like domains (IgFLN1-24). Dimerization of filamin through IgFLN24 results in a flexible parallel homodimer that can induce high-angle branching of actin filaments, promoting the formation of orthogonal actin networks. With more than 50 binding partners, filamin A plays an integral role in anchoring cell-surface receptors to the actin cytoskeleton and localizing cytoplasmic signaling proteins. In platelets, the protein tyrosine kinase Syk binds at IgFLN5,7 whereas the GPIbα subunit recognition site in filamin A resides at IgFLN17.8 The cytoplasmic residues 557 to 575 on the GPIbα subunit constitute the GPIbα/V/IX–filamin interaction site.9 In this issue, Cranmer and colleagues demonstrate that GPIbα–filamin binding is required for maintaining the mechanical integrity of the platelet membrane during platelet adhesion to VWF under shear flow (see figure).

Using a transgenic mouse model with human GPIbα (hGPIbα)–expressing platelets, Cranmer et al engineered the substitution of 2 residues in the cytoplasmic domain (Phe568Ala and Trp570Ala) of hGPIbα to disrupt the hGPIbα–filamin linkage (hGPIbαFW). The authors demonstrate that platelet recruitment to human VWF under shear remained unchanged in the absence of the hGPIbα–filamin linkage, suggesting that the GPIbα–filamin A interaction is dispensable for the intrinsic VWF binding function of
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