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Cytokine-free rapid red cell regeneration

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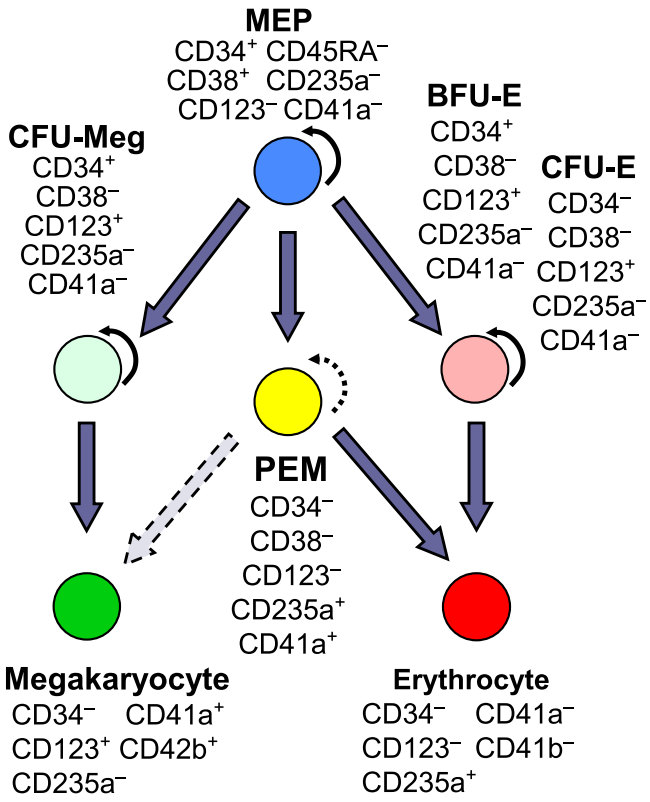
In this issue of *Blood*, Belay et al present evidence of human bipotent erythroid-megakaryocyte precursor cells that can bypass the burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) stages and directly and rapidly differentiate into either erythrocytes or megakaryocytes.[1]

The properties of the cells they describe resemble those of cells previously observed during stress erythropoiesis in mice, also initially reported in *Blood*,[2,3] suggesting the existence of a novel cellular response to erythropoietic stress in both mice and humans.

The studies reported by Belay et al are an extension of their work pursued for many years on expansion of hematopoietic stem progenitor cells (HSPCs) ex vivo using a novel engineered artificial cell growth switch (CGS) receptor consisting of the Mpl intracellular signaling domain fused to a dimerizable receptor for a small drug molecule referred to as a chemical inducer of dimerization (CID). Previous work using the CGS system showed that cell expansion was limited to primitive erythroid T and B cells, megakaryocytes, and platelets, with minimal effects on primitive HSPCs. Belay et al hypothesize that the lack of expansion of primitive HSPCs is due to low levels of CGS signaling from the CGS receptor, and therefore attempt to improve CGS signaling by introducing point mutations at locations within the Mpl signaling domain involved in degradation of the human Mpl receptor. These engineered hyperactive Mpl constructs increase the sensitivity and responsiveness to an exogenous CID when introduced into human adult or cord blood $CD34^+$ cells and significantly improve the expansion of $CD34^+$ cells and maintenance of colony-forming cells. No evidence is presented regarding expansion of the most primitive hematopoietic stem cells.



The PEM population in the hierarchy of human hematopoiesis. CFU-Meg, CFU-megakaryocyte. See Figure 7 in the article by Belay et al that begins on page 1025.

Through careful and detailed efforts to characterize the CGS-expanded cell populations, Belay et al identified a novel cell type.[1] A population of $CD235^+/CD41^+$ cells was expanded >70-fold and constituted up to 13% of cultured CGS-expanded cells. This $CD235^+/CD41^+$ cell population contained few colony-forming cells but could rapidly differentiate (within 48 hours) into erythrocytes and megakaryocytes. Differentiation was not dependent on stem cell factor or associated with increased cell numbers. Low numbers of these $CD235^+/CD41^+$ bipotent precursors were also observed in unexpanded cord blood cell populations. The properties of these cells closely resemble those of a $Ter119^+/4A5^+$ population designated "precursors for erythrocytes and megakaryocytes" (PEMs) previously observed in erythropoietically stressed mice.[2,3] Belay et al propose an alternative pathway of human erythroid differentiation under conditions of erythropoietic stress that allows PEM cells derived from bipotent megakaryocyte-erythrocyte progenitors (MEPs) to bypass the BFU-E and CFU-E stages and directly differentiate into erythrocytes (see figure). This human model is analogous to the previously proposed murine model for stress erythropoiesis stating that the pathway of orderly unilineage differentiation[4] during steady-state conditions was altered during stress erythropoiesis by generation of rapidly

differentiating PEMs from both MEPs and common myeloid progenitors (CMPs) to achieve speedy regeneration of erythroid cells.[3] Confirmation of the human model awaits the further analysis of PEM cells in vivo in humans and demonstration of the importance of these cells in the human erythropoietic stress response. Due to the difficulties of conducting such studies in humans, studies in primate model systems that are very similar to humans could be valuable. Minor differences in the human and murine models will require further clarification. In the human model, the PEM population is derived directly from the MEP, whereas the murine model suggests that PEMs may be derived from both MEPs and CMPs. In addition to the PEM population, the authors also described a population of macrophages associated with differentiating erythroblasts that was expanded in a CID-independent manner. Further experiments will be required to characterize the mechanism of macrophage expansion and the relationship of these cells to the differentiating erythroblasts.

The ability of PEM cells to directly and rapidly differentiate into erythrocytes suggests that the erythroid stress differentiation pathway might be involved in the generation of erythrocytes containing increased levels of fetal hemoglobin (HbF) (F cells). In human and nonhuman primate models, HbF and F cells are normally present at low levels but increase during erythropoietic stress.[5,6] Because more primitive cells are programmed to express higher levels of HbF than more differentiated cells, increased HbF expression can result from commitment to differentiation at more primitive stages of the erythroid differentiation pathway.[7] Interestingly, Belay et al observed, as predicted, a higher ratio of γ-globin in PEMs than in erythroid cells. Further understanding of the mechanisms controlling HbF and F cell numbers could impact the treatment of sickle cell disease and β-thalassemia, where increased HbF and F cell numbers are beneficial.

One of the potentially promising applications of the current findings is to exploit the expansion of erythroid cells from bipotent PEM progenitors free of exogenous cytokines in culture using the CGS-CID system as described by Belay et al.[1] Currently, scaling up to produce 1 unit of packed cultured red blood cells (RBCs) from human peripheral blood $CD34^+$ cells is feasible but is limited by excessive production costs, which are largely due to the requirement for exogenous cytokines.[8] Therefore, exogenous cytokine–free ex vivo–cultured human RBC production would enable a significant reduction in costs. Certainly, much more work will need to be done to demonstrate that the purified PEM-derived RBCs not only are functional in vivo and behave similar to native RBCs but also are free of contaminating myeloid progenitors. Of note, as proof of principle, cultured human RBCs have already been generated and transfused in a single human subject.

In conclusion, Belay et al have identified a human bipotent erythroid-megakaryocytic precursor cell with the ability to rapidly differentiate into both erythrocytes and megakaryocytes. The qualities of this human bipotent cell, analogous to those of murine PEM cells arising during stress erythropoiesis, strongly suggest that the erythroid differentiation pathway is likely governed in a similar manner in response to stress erythropoiesis in both mice and humans. The identification of this new erythroid-biased bipotent population may reveal a cost-effective means of generating cultured human RBCs for possible blood transfusions in the future.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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VWF and complement

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In this issue of *Blood*, Feng and coworkers present data suggesting a role for von Willebrand factor (VWF) in the proteolytic inactivation of complement C3b by factor I (CFI).[1] Whereas smaller VWF multimers, especially dimers, tetramers, and hexamers, enhance C3b inactivation by CFI, large and unusually large VWF multimers are devoid of this cofactor activity and, therefore, they enhance complement activation by the alternative pathway C3 convertase, C3bBb.

When a severe deficiency of VWF-cleaving protease, either caused by autoantibodies inhibiting its activity or by a constitutional defect without circulating inhibitors, was first reported in a series of patients with sporadic or familial thrombotic thrombocytopenic purpura (TTP), respectively, a differential diagnosis between TTP and atypical hemolytic uremic syndrome (HUS) seemed to become possible.[2] In contrast to patients clinically diagnosed with TTP, those with a diagnosis of atypical HUS showed normal or only mildly decreased activity of the VWF-cleaving