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Stress erythropoiesis: new signals and new stress progenitor cells

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Abstract

Purpose of review—Acute anemic stress induces a physiological response that includes the rapid development of new erythrocytes. This process is referred to as stress erythropoiesis, which is distinct from steady state erythropoiesis. Much of what we know about stress erythropoiesis comes from the analysis of murine models. In this review, we will discuss our current understanding of the mechanisms that regulate stress erythropoiesis in mice and discuss outstanding questions in the field.

Recent findings—Stress erythropoiesis occurs in the murine spleen, fetal liver and adult liver. The signals that regulate this process are Hedgehog, bone morphogenetic protein 4 (BMP4), stem cell factor and hypoxia. Recent findings show that stress erythropoiesis utilizes a population of erythroid-restricted self-renewing stress progenitors. Although the BMP4-dependent stress erythropoiesis pathway was first characterized during the recovery from acute anemia, analysis of a mouse model of chronic anemia demonstrated that activation of the BMP4-dependent stress erythropoiesis pathway provides compensatory erythropoiesis in response to chronic anemia as well.

Summary—The BMP4-dependent stress erythropoiesis pathway plays a key role in the recovery from acute anemia and new data show that this pathway compensates for ineffective steady state erythropoiesis in a murine model of chronic anemia. The identification of a self-renewing population of stress erythroid progenitors in mice suggests that therapeutic manipulation of this pathway may be useful for the treatment of human anemia. However, the development of new therapies will await the characterization of an analogous pathway in humans.

Keywords

anemia; bone morphogenetic protein 4; erythropoiesis; Hedgehog; hypoxia

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Introduction

Steady state erythropoiesis is primarily homeostatic, producing new erythrocytes at a constant rate. For the most part, it is restricted to the bone marrow. In contrast, tissue hypoxia caused by anemia or during fetal development results in the activation of a physiological stress response designed to increase oxygen delivery to tissues. Increased erythropoiesis is a key component of this response [1]. At these times, stress erythropoiesis predominates, which is capable of rapidly generating large numbers of erythrocytes [2]. In the mouse, stress erythropoiesis occurs in the in adult spleen and liver [3–5] and during fetal development in the fetal liver [6]. Although early work in the field identified the murine spleen as the primary site of erythropoiesis during recovery from experimentally induced anemia, it was proposed that stress erythropoiesis represented an enhanced version of steady state erythropoiesis [7,8]. These studies suggested that as anemia progresses, tissue hypoxia increases and leads to the induction of erythropoietin (Epo) expression in the kidney [9]. The increase in serum Epo concentration drives the expansion and differentiation of bone marrow erythroid progenitors [10,11]. Some of these progenitors would then migrate to the spleen and finish their differentiation. This model is based on two ideas. The first idea is that Epo is the primary signal that drives stress erythropoiesis and the second is that the erythroid progenitors that respond to anemic stress are the same as the bone marrow steady state erythroid progenitors. Epo does play a key role in this process, primarily promoting the terminal differentiation of stress erythroid progenitors [12,13]. This role in stress erythropoiesis was recently reviewed in this series [1]. In this review, we will focus on earlier events in the response and propose a more comprehensive model for stress erythropoiesis, which is supported by recent work showing that stress erythropoiesis relies on a population of stress erythroid progenitor cells that are distinct from bone marrow steady state erythroid progenitors [2]. The development, expansion and differentiation of these progenitors is regulated in part by signals not previously associated with adult erythropoiesis: bone morphogenetic protein 4 (BMP4) and Hedgehog, which act in concert with signals previously associated with stress erythropoiesis – Epo, stem cell factor (SCF) and hypoxia.

Bone morphogenetic protein 4 regulates the expansion of stress erythroid progenitors resident in the spleen during the recovery from acute anemia

Our work on stress erythropoiesis started with the analysis of the murine *flexed-tail (f)* mutation. *f/f* mutant mice exhibit a fetal–neonatal anemia that resolves about 2 weeks after birth [14–19]. Despite having near normal steady state erythropoiesis, adult *f/f* mice are slow to recover from phenylhydrazine (PHZ)-induced acute hemolytic anemia [20]. The delayed recovery observed in *f/f* mice is caused by a defect in the expansion of erythroid progenitors (BFU-E) in the spleen [2]. Bone marrow erythropoiesis is unaffected. Careful analysis of erythroid progenitors showed that spleen BFU-E differed from bone marrow BFU-E. They form larger colonies, much faster (5 vs. 7 days) than their bone marrow counterparts. Furthermore, unlike bone marrow BFU-E, which require both Epo and a burst-promoting signal, spleen BFU-E formed colonies in media containing only Epo. This observation is similar to human fetal BFU-E that can also form colonies with Epo alone [21], which is consistent with the idea that spleen erythroid progenitors are distinct from bone marrow erythroid progenitors and may be more similar to fetal erythroid progenitors. Cloning of the *f* mutant locus showed that it encoded Smad5 [2,22], a receptor activated Smad that functions downstream of the receptors for BMPs 2, 4 and 7 [23]. Subsequent analysis showed that BMP4 induced the expansion of BFU-E in the spleen during the recovery from acute anemia. Together, these data suggested a new model for stress erythropoiesis in which BMP4 drives the expansion of a population of specialized stress erythroid progenitors that are resident in the spleen, which we termed ‘stress BFU-E’. These progenitors exhibit ideal

properties of a stress erythroid progenitor in that they rapidly produce large numbers of mature erythrocytes at times of acute stress.

In addition to BMP4, two other signals drive the expansion of stress erythroid progenitors in the spleen. Early work on two murine mutants, *dominant white spotting* (*W*) and *steel* (*Sl*), which encode the Kit receptor [24,25] and its ligand SCF [26–28], respectively, demonstrated a role for the Kit/SCF signaling pathway in stress erythropoiesis. Although this early work suggested that the defect in stress erythropoiesis in *W* and *Sl* mutant mice was a corollary of the macrocytic anemia present in these mice [29], analysis of *W* mutant mice showed a defect in the expansion of stress BFU-E in the spleen during the recovery from anemia [30]. Kit receptor signaling activates numerous downstream pathways and plays a wide-ranging role in regulating hematopoiesis. Recent work dissecting the response downstream of Kit showed that specific signaling pathways regulated by the phosphorylation of tyrosine 567 of Kit receptor are required for the recovery from acute anemia [31•]. Although BMP4-dependent and SCF-dependent signals are required for the expansion of stress BFU-E, they are not sufficient to recapitulate *in vitro* the expansion of stress BFU-E observed *in vivo* during the recovery from acute anemia. The full response requires hypoxic (1% O_2) culture conditions. Hypoxia potentiates the response of stress erythroid progenitors to BMP4 and alters the response to SCF. Under normal atmospheric O_2 (20%) conditions SCF modestly increases the size of the bursts, but in the presence of low O_2 (1%) SCF significantly increases the number of stress BFU-E and greatly increases the burst size [30]. The mechanism by which hypoxia alters the response of stress BFU-E to SCF and BMP4 is not understood. Further insights into this mechanism could aid in the development of drugs that could potentially improve stress erythropoiesis in clinical situations. One key aspect of the regulation of BMP4-dependent stress erythropoiesis pathway is this requirement for hypoxia. It functions at two levels to limit stress erythropoiesis to times of anemic stress. Not only is hypoxia required for the maximal response of stress progenitors to BMP4 and SCF [30], hypoxia also regulates the induction of BMP4 expression in the liver and spleen [32]. Hypoxia-inducible factor-2α regulates BMP4 expression by binding to two response elements in the *BMP4* gene.

Hedgehog signaling regulates the maintenance of the stress progenitor pool in the spleen

Acute anemia leads to the complete mobilization of stress erythroid progenitors in the spleen, so there must be a mechanism by which new stress erythroid progenitors are replenished following recovery [33•]. Although bone marrow erythroid progenitors do not respond to BMP4, SCF and hypoxia dependent signals in a manner similar to spleen stress progenitors, transplant experiments showed that bone marrow progenitor cells that migrate to the spleen adopt the stress erythroid progenitor fate. This observation suggested that the spleen microenvironment contained a signal that induced cells to become stress erythroid progenitors. This situation is similar to chondrocyte development during murine embryogenesis. In this case, BMP4 induces somitic mesoderm cells to differentiate into chondrocytes, but prior to differentiation Hedgehog signaling is required to make these cells competent to respond to BMP4 [34]. Similarly, the development of new stress erythroid progenitors in the spleen requires Hedgehog signaling to induce bone marrow progenitor cells to adopt the stress progenitor fate, which allows them to respond to BMP4, SCF and hypoxia during the recovery from acute anemia. When Hedgehog signaling is blocked by the deletion of the Hedgehog receptor *smoothened* (*Smo*) in adult mice, stress erythroid progenitors fail to develop in the spleen following recovery from acute anemia. Furthermore, culturing bone marrow cells with Hedgehog induces the development of stress BFU-E. These experiments established a role for Hedgehog signaling in stress erythropoiesis [33•]. The focus of current work is to identify the targets of Hedgehog

signaling that specify the stress erythroid fate. During chondrocyte development Sonic Hedgehog induces the expression of two transcription factors Nkx3.2 and Sox9, which promote cartilage development in a BMP4-dependent manner [35,36]. It is not clear if these factors or other members of the Nkx or Sox families function in stress erythropoiesis.

Bone morphogenetic protein 4-dependent stress erythropoiesis utilizes a self-renewing population of erythroid-restricted stress progenitors

On the basis of these earlier studies, a model for stress erythropoiesis emerged in which stress erythroid progenitors are resident in the spleen. Anemic/hypoxic stress induces the expression of BMP4, which acts in concert with SCF, hypoxia and Epo to promote the rapid expansion and differentiation of new erythrocytes. In the postrecovery period, bone marrow cells migrate into the spleen and in response to Hedgehog adopt the stress erythroid fate, which replenishes the pool of stress erythroid progenitors (Fig. 1). Murine adult bone marrow steady state erythroid progenitors are characterized by the expression of CD71 and Ter119 cells [11,37]. In contrast, spleen stress progenitors, expanded *in vitro*, were $Kit^{+}CD71^{+}Ter119^{+}$, which is consistent with the idea that stress erythroid progenitors are distinct from steady state erythroid progenitors [30]. Further analysis of these progenitors required a new experimental system, bone marrow transplant (BMT), which takes advantage of the observation that in order to avoid lethal anemia, new erythrocytes must be made prior to engraftment of donor stem cells. These erythrocytes are produced by short-term radioprotective cells, which also produce platelets and neutrophils in the immediate posttransplant period [38,39]. BMT assays done with donor cells that were defective in BMP4 signaling exhibited a defect specifically in erythroid recovery with no defects in the generation of platelets and neutrophils. Therefore, erythroid short-term radioprotection is BMP4-dependent stress erythropoiesis [40••]. One of the advantages of the BMT assay is that donor cells can be followed by using different alleles of CD45 cells in congenic donors and recipients. Analysis of donor-derived cells in the spleen showed that the $Kit^{+}CD71^{+}Ter119^{+}$ population of stress erythroid progenitors could be resolved into three distinct populations (Fig. 2). Fluorescence-activated cell sorting analysis showed that the three populations referred to as populations I, II and III had distinct erythroid potential. Population I cells, which are present in the spleens of untreated mice, contained all the stress BFU-E. Population II cells gave rise to CFU-E. Whereas population III cells did not form colonies and were approximately 50% benzidine positive, which is consistent with these cells being the most mature. Analysis of the three populations during recovery suggests a temporal relationship in which population I cells differentiate into population II cells, which in turn generate population III cells. Further analysis of population I yielded the surprising result that a large percentage of these cells were also Sca1 positive. The presence of hematopoietic stem cell (HSC) markers [41–45], Kit and Sca1, on the surface of population I cells suggested that they might be multi-potential and have the potential to self-renew. Surprisingly, transplanting purified population I cells into lethally irradiated secondary recipients rescued erythropoiesis, but without contribution to other lineages. Furthermore, donor-derived cells isolated from the spleens of secondary transplants were able to rescue erythropoiesis in tertiary transplants with similar efficiency to that observed in the secondary transplants. Analysis of secondary transplants showed that donor population I cells generated an initial wave of erythropoiesis that maintained the survival of the recipient mice. Recipient HSCs that had survived the radiation treatment then repopulated the mice and replaced the donor-derived erythrocytes. Surprisingly, treatment of these mice with PHZ to induce anemia led to the generation of donor-derived erythrocytes, which suggests that the donor population I cells have established a durable stress erythroid compartment in the recipient mice that is capable of responding to subsequent anemic challenges. Taken together, these data demonstrate that erythroid short-term radioprotection utilizes self-

renewing erythroid-restricted stress progenitor cells. These results also raise several interesting questions. What signals regulate the self-renewal of population I cells and what signals regulate the commitment to differentiation? Furthermore, what is the mechanism that restricts these progenitors to the erythroid lineage?

Activation of the bone morphogenetic protein 4-dependent stress erythropoiesis pathway alleviates the anemia in a mouse model of anemia induced by chronic inflammation

Both acute hemolytic anemia caused by PHZ injection and anemia following BMT are excellent experimental models of acute anemia. In clinical settings, chronic anemia and anemia associated with chronic inflammation, also known as the anemia of chronic disease (ACD), are much greater problems [46,47]. Because acute anemia leads to the complete mobilization of stress progenitors in the spleen, it was unclear how this pathway would function in chronic anemia. However, recent work by Millot *et al.* [48•] addressed this question and identified a role for stress erythropoiesis in the response to chronic anemia. They used a murine model for chronic inflammation: the zymosan-induced general inflammation (ZIGI) mouse, which causes severe generalized inflammation that leads to long lasting anemia [49]. Bone marrow erythropoiesis is suppressed in the ZIGI mice, but treatment with Epo partially alleviates the anemia. However, Epo does not increase bone marrow erythropoiesis, but rather induces stress erythropoiesis in the spleen. Bone marrow BFU-Es are inhibited by interferon-γ (IFNγ), which is highly expressed in inflammatory disease. In contrast, spleen stress BFU-Es were not affected by IFNγ treatment. Epo induces the expression of BMP4 by spleen macrophages. Whether this induction is direct or indirect is not clear. The increased expression of BMP4 leads to an expansion of stress BFU-E and increased erythropoiesis. Of interest, these experiments show that unlike acute anemia in which BMP4-dependent stress erythropoiesis is induced by acute hypoxia, in this model of chronic anemia the pathway is inhibited despite the severe anemia. Epo injection is required to activate the pathway. The differential response of the BMP4-dependent stress erythropoiesis pathway to acute vs. chronic anemia is also highlighted by analysis of mice carrying a conditional allele of the *Epo* gene [50•]. Deletion of the *Epo* gene in adult mice leads to severe Epo-deficient anemia. Despite the anemia, $Epo^{\Delta/\Delta}$ mice recovered from PHZ-induced acute anemia with the same kinetics as controls. On the basis of these observations, further studies will be needed to understand the mechanisms that regulate stress erythropoiesis in chronic anemia.

Is there an equivalent bone morphogenetic protein 4-dependent pathway in human stress erythropoiesis?

Experiments using the ZIGI mouse model suggest that manipulation of the BMP4-dependent stress erythropoiesis pathway could be used as a potential treatment for ACD. However, any discussion about potential therapies is purely speculative because an analogous pathway has not been identified in humans. The analysis of stress erythropoiesis in humans is difficult because direct experiments to examine the mechanisms that regulate stress erythropoiesis are not possible and analysis of erythropoiesis in patients with severe anemia can be complicated by indirect effects of disease pathology. Despite these difficulties, some general themes have been identified in human stress erythropoiesis. Human stress erythropoiesis often exhibits properties of fetal erythropoiesis. Fetal erythrocyte characteristics and antigens as well as the expression of fetal hemoglobin (HbF) are observed during the recovery from erythropoietic stress [51,52]. These characteristics are observed following BMT [53–55], in acute anemia syndromes like transient erythro-blastopenia of childhood [56,57] and in some patients with thalassemia and sickle cell anemia [58–62]. Similar effects

are observed in nonhuman primates treated with PHZ [63–65]. A few studies have reported observations that support a possible connection between the murine BMP4-dependent stress erythropoiesis and the stress erythropoiesis in humans. Analysis of peripheral blood and bone marrow progenitors from sickle cell anemia patients identified a population of progenitor cells that express CD34 and glycophorin A (GPA) on their surface. These cells also express CD71 and KIT. These progenitor cells give rise to a higher proportion of HbF^+ F cells than normal bone marrow progenitors. The production of F cells is also increased when cells are grown at 5% O_2 compared with ambient 20% O_2 [66,67]. The murine bone marrow population that gives rise to stress BFU-E in the spleen following BMT is the short-term reconstituting HSCs, which are $CD34^+Kit^+Sca1^+Lin^-$ cells [45]. It is interesting to speculate that the $CD34^+GPA^+CD71^+Kit^+$ cells may be the human equivalent of the murine stress progenitors we observed during the recovery from BMT; however, further work will be needed to establish any connection between these ‘stress’ progenitors observed in patients and the BMP4-dependent stress erythropoiesis pathway.

Conclusion

The identification of the BMP4-dependent stress erythropoiesis pathway and the characterization of the stress erythroid progenitor cell populations and the signals that regulate this process are important step in understanding how anemic stress alters erythropoiesis. In the murine system, this model will serve as a conceptual framework for the reanalysis of previous work and as a guide for future studies. Although no data exists to demonstrate that human stress erythropoiesis utilizes similar signals, the work done in mice has established an excellent starting point for future experiments using human progenitors. The goal of this work is to increase our understanding of human stress erythropoiesis to such an extent that targets for therapeutic intervention could be identified and tested in the clinic.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 197).

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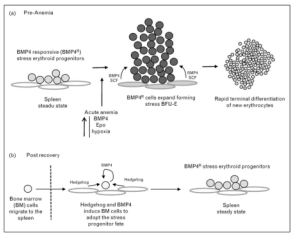

Figure 1. Model of the bone morphogenetic protein 4 stress erythropoiesis pathway

(a) Activation by acute anemia. (b) Postrecovery replenishment of stress progenitors. BFU-E, burst forming unit-erythroid; BM, bone marrow; BMP4, bone morphogenetic protein 4; Epo, erythropoietin; SCF, stem cell factor.

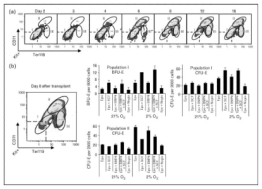

Figure 2. Three distinct populations of stress erythroid progenitors develop in the spleen during the recovery from bone marrow transplant

(a) Flow cytometry analysis of spleen cells on the indicated days after transplant. The cells are gated on Kit^+ cells and the expression of CD71 and Ter119 is analyzed. (b) Populations I, II and III were sorted on day 8 after transplant and plated for burst forming unit-erythroid (BFU-E) and colony forming unit-erythroid (CFU-E). Population II cells did not give rise to BFU-E and Population III cells failed to form colonies of any type. BMP4, bone morphogenetic protein 4; Epo, erythropoietin; SCF, stem cell factor. Adapted from [40••].