Experimental Hematology

Experimental Hematology 2020;91:10–21

REVIEW ARTICLE

The erythroblastic island niche: modeling in health, stress, and disease

Alisha May, and Lesley M. Forrester

Centre for Regenerative Medicine, Institute for Regeneration and Repair, University of Edinburgh, Edinburgh, Scotland, UK

(Received 27 July 2020; revised 2 September 2020; accepted 3 September 2020)

Erythropoiesis is one of the most demanding processes in the body, with more than 2 million red blood cells produced every second. Multiple hereditary and acquired red blood cell disorders arise from this complex system, with existing treatments effective in managing some of these conditions but few offering a long-term cure. Finding new treatments relies on the full understanding of the cellular and molecular interactions associated with the production and maturation of red blood cells, which take place within the erythroblastic island niche. The elucidation of processes associated within the erythroblastic island niche in health and during stress erythropoiesis has relied on in vivo modeling in mice, with complexities dissected using simple in vitro systems. Recent progress using state-of-the-art stem cell technology and gene editing has enabled a more detailed study of the human niche. Here, we review these different models and describe how they have been used to identify and characterize the cellular and molecular pathways associated with red blood cell production and maturation. We speculate that these systems could be applied to modeling red blood cell diseases and finding new druggable targets, which would prove especially useful for patients resistant to existing treatments. These models could also aid in research into the manufacture of red blood cells in vitro to replace donor blood transfusions, which is the most common treatment of blood disorders.

The erythroblastic island, first visualized by Marcel Bessis in 1958, is the site of erythropoiesis in mammals [1]. Erythroblastic islands are situated predominantly in the bone marrow during steady-state erythropoiesis, but expand in the fetal liver and adult spleen during stress erythropoiesis, when there is a rapid production of erythrocytes in response to inflammation and anemia [1–4]. The island consists of a central macrophage (erythroblastic island [EBI] macrophage), surrounded by developing erythroblasts, which were hypothesized to supply ferritin to the developing erythroblasts for hemoglobin synthesis [5,6]. Additional functions of the central macrophage have since been elucidated, confirming its role as a supportive "nurse" cell during erythropoiesis. EBI macrophages support erythroblast differentiation through cell–cell contact and secreted supportive factors, promoting the maturation and enucleation of erythroid cells and the phagocytosis of their expelled nuclei [7–10].

Although there have been extensive studies into the hematopoietic stem cell (HSC) bone marrow niche microenvironment, the erythroblastic island niche (EBI niche) has been the focus of a relatively small number of research groups [11–13]. In contrast to the vast array of cell types implicated in the HSC niche, the EBI niche appears to be relatively simple, with no other cell type being implicated to work in concert with EBI macrophages [14]. This offers the EBI as a unique and streamlined niche to study red blood cell (RBC) development in health and disease, and the development of model systems is relatively straightforward.

Offprint requests to: Lesley Forrester, Centre for Regenerative Medicine, Institute for Regeneration and Repair, University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU, Scotland, UK; E-mail: L.Forrester@ed.ac.uk

https://doi.org/10.1016/j.exphem.2020.09.185

Anemia, the most prevalent RBC disorder, is crudely defined as a condition in patients who lack adequate numbers of healthy RBCs and is diagnosed by a low blood hemoglobin (Hb) concentration [15]. Anemia affects approximately 1.62 billion people worldwide, accounting for 8.8% of the total worldwide disease burden [16,17]. RBC disorders represent a broad spectrum of conditions, spanning inherited disorders, such as thalassemia and sickle cell anemia, to acquired disorders, such as polycythemia vera and paroxysmal nocturnal hemoglobinuria (PNH), with mild to fatal clinical outcomes [18–22].

Current treatments for RBC disorders include blood transfusions and iron chelation therapy to counteract iron accumulation in patients requiring repeated transfusion, or iron supplements for iron-deficiency anemia [23,24]. Several drugs that stimulate erythropoiesis have been approved; one such drug is erythropoietin (EPO), which is used routinely to treat anemias associated with chronic kidney disease and cancer [25–27]. Rarer RBC disorders, such as PNH, have fewer effective treatment options, and these are often prohibitively expensive. Eculizumab, for example, is a monoclonal antibody against terminal complement protein C5 that is used to reduce hemolysis and stabilize hemoglobin levels. Not only is it widely reported as one of the world's most expensive drugs at a cost of around $400,000 per year of treatment, but only 20% of patients reach normal hemoglobin values and 40% remain anemic [28,29]. Some RBC disorders, including PNH and acquired aplastic anemia, can be effectively cured by bone marrow transplantation [30,31], but this procedure is associated with significant morbidity and mortality rates because of the requirement for preconditioning, which ablates the hematopoiesis of the host [31,32]. There is a clear clinical need for the development of new therapies to treat RBC disorders.

A full understanding of the cellular and molecular pathways involved in the EBI niche and how these contribute to stress erythropoiesis and disease would allow for the development of new therapies to treat RBC disorders. Early models of the EBI niche provided insights into its structure and organization and were later followed by more sophisticated genetic models that reflected the roles of individual genes in the niche in both animal models and humans. Here, we review the in vivo and in vitro models that have been used to study the EBI niche. We discuss the advantages and disadvantages of each (Table 1) and summarize some of the key advances that have been made using these models in our understanding of steady state, stress, and disease erythropoiesis (Figure 1).

Erythropoiesis and the EBI

In the first stage of RBC production, HSCs sequentially differentiate to common myeloid progenitor, megakaryocyte–erythroid progenitor, burst-forming unit–erythroid (BFU-E), and colony-forming unit–erythroid (CFU-E) progenitor cells [33–36]. In the second stage, CFU-E progenitor cells differentiate through the morphologically distinct nucleated precursors proerythroblast, basophilic erythroblast, polychromatic erythroblast, and orthochromatic erythroblast [37]. As the differentiating erythroid cells mature, nuclear

Table 1. Model systems used to study the erythroblastic island niche

Species	Model	Advantages	Disadvantages	References
Mouse	Imaging and isolation of intact EBIs	Visual	Static system	1,2,50
Mouse	Reconstitution of islands in vitro	Allows the study of direct cell–cell interactions in a dynamic manner Cheap and simple	Not possible to investigate possible interactions with other cell types	42,55,57–60
Mouse	Macrophage depletion in vivo (e.g., clodronate)	Easy to administer Can be performed on different genetic models	Broad macrophage depletion	63,65
Mouse	Genetic models (e.g., lineage-specific knockout and depletion)	Allows for dissection of the role of specific cell populations and genes in vivo	Costly Complex	56,58,74,77,79–81,83,90,100
Human	Imaging and isolation of intact EBIs	Visual	Static system Difficult to obtain primary tissue	45
Human	Reconstitution of EBIs in vitro using HSPC-derived cells	Macrophage/erythroid interactions can be studied Relatively cheap and simple system Macrophages and erythroid cells differentiate in concert	Reliant on repeated donations Does not investigate involvement of other cell types	102–104
Human	Reconstitution of EBIs in vitro using monocyte-derived macrophages	Macrophage/erythroid interactions can be studied Relatively cheap and simple system	Not conducive to genetic manipulation Reliant on repeated donations	44,105,106
Human	Reconstitution of EBIs in vitro using iPSC-derived macrophages	Macrophage/erythroid interactions can be studied Relatively cheap and simple Limitless resource Genetically manipulatable Disease-specific modeling possible	May not completely recapitulate in vivo EBIs	8

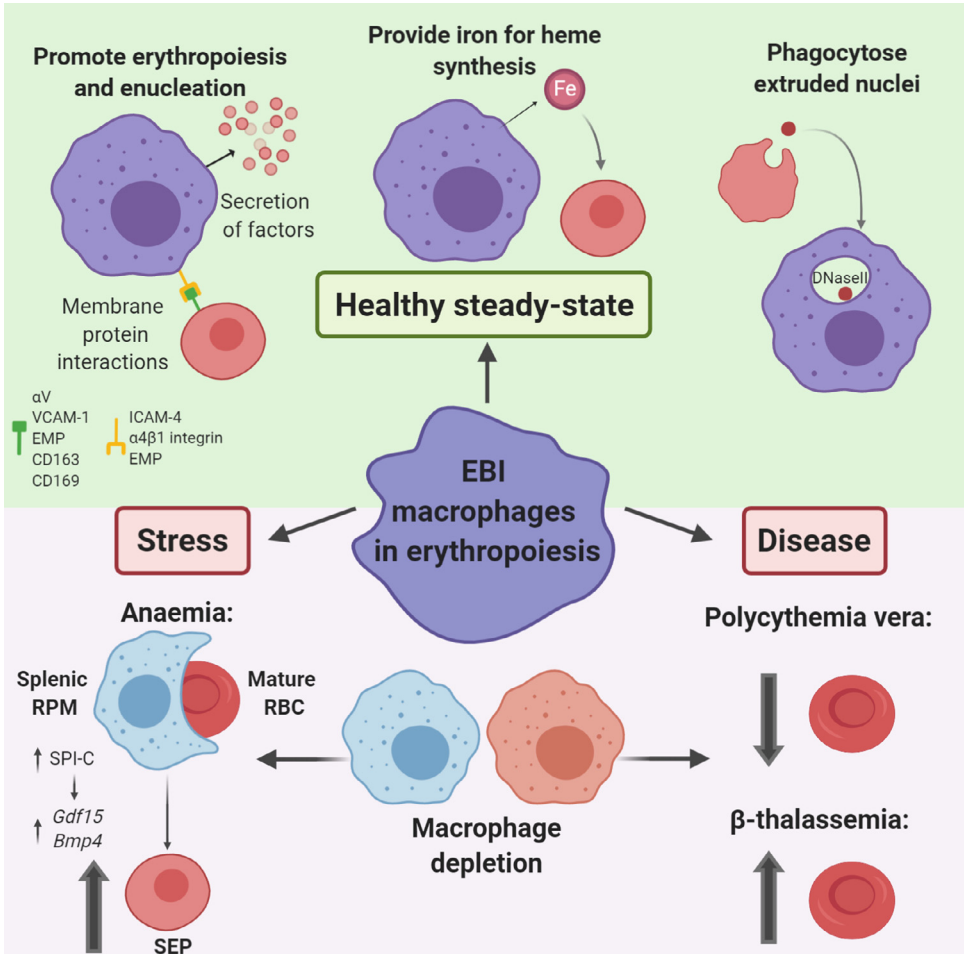

Figure 1. Role of EBI macrophages in erythropoiesis. In healthy steady-state erythropoiesis, EBI macrophages support and promote erythropoiesis via membrane–protein interactions and the secretion of factors and by providing iron for heme synthesis. During terminal differentiation, EBI macrophages phagocytose nuclei extruded from differentiating erythroid cells with DNase11 associated with its degradation. In models of stress erythropoiesis, macrophage depletion led to the phagocytosis of mature RBCs by splenic red pulp macrophages, which increased expression of SPI-C, Gdf15, and Bmp4, resulting in an increase in the proliferation of stress erythroid progenitors. Macrophage depletion led to the normalization of RBC numbers in disease models of polycythemia vera and *β*-thalassemia by decreasing and increasing red blood cells, respectively. Macrophages associated with the EBI in the steady state (*purple*) are likely to be different from those in stress and diseased conditions (*blue*).

chromatin is condensed and cytoskeletal remodeling occurs in preparation for nuclear expulsion [38,39].

The pro-erythroblast-to-orthochromatic stages of differentiation occur within the EBI. Macrophage–erythroblast adhesion molecules function to promote erythroblast proliferation and are highly expressed in pro-erythroblasts, with expression progressively lost by the orthochromatic erythroblast stage [40–42]. The central macrophage secretes cytokines that promote the enucleation of erythroblasts and provides iron for heme synthesis [6,8,43,44]. The composition of EBIs varies slightly across species, with rat EBIs containing consistently around 10 erythroblasts per island compared with much more variable human EBIs, where 5 to 30 erythroblasts can be found surrounding the central macrophage [45,46].

At the final terminal differentiation stage, the nucleus is expelled from the orthochromatic erythroblast and phagocytosed by the EBI macrophage [47]. The resulting reticulocyte expels any remaining organelles and enters circulation [38]. Considerable membrane remodeling then takes place to generate fully mature, biconcave erythrocytes [48,49].

Modeling the EBI niche using animal models

Isolation of intact EBIs

Early investigations into the structure and organization of EBIs involved careful isolation and study of rodent bone marrow using various types of microscopy [50]. The first image of an EBI, obtained by Bessis in 1958,

was acquired by examining bone marrow preparations using phase-contrast microscopy [1]. Light and electron microscopy were later used to construct three-dimensional reconstructions of rat bone marrow, revealing distinct in situ EBIs in which erythroblasts underwent maturation in close association with macrophages [2]. After these initial morphological assessments of the central mononuclear cells present in EBIs as macrophages, F4/80 antibody staining confirmed that resident macrophages in mouse bone marrow formed the EBIs [51]. Light and electron microscopy has also been used to illustrate that EBIs are not spatially restricted within the bone marrow, and their composition is altered depending on their location. For example, EBIs adjacent to sinusoids are enriched for orthochromatophilic erythroblasts, while nonadjacent EBIs are enriched in pro-erythroblasts, suggesting a mechanism in which islands migrate to sinusoids as erythroid differentiation progresses [52]. Although the information gleaned from microscopy studies tends to present EBIs as static structures, the fact that they migrate indicates that they are actually quite dynamic and it is likely that the cell–cell interactions change over time. In tandem with microscopy studies, isolated EBIs were further characterized via antibody staining [53]. EBIs were found to range from 5 to 100 cells, and the majority of isolated islands contained at least one F4/80$^+$ central macrophage [53]. Isolation of intact EBIs from different hematopoietic tissues and subsequent analysis of known EBI macrophage cell surface markers revealed them to be a heterogeneous macrophage population [54–56]. Mouse and rat EBIs heterogeneously express VCAM-1, F4/80, and CD169, while CD163 is expressed only by rat macrophages [54]. The study of intact EBIs provided visual data for the composition of islands and is useful for characterizing the component phenotypes of the EBI niche in vivo but it has limitations. It is a static system and cannot be used to dissect the molecular mechanisms associated with its function (Table 1).

In vitro reconstitution of EBIs

As well as ex vivo culture of EBIs harvested from rodents, the reconstitution of EBIs in vitro through the co-culture of erythroblasts and macrophages was first proposed in 1979, and is now a widely employed method [9,42,57–60]. Through studies using reconstituted EBIs, it was observed that macrophages support erythroid differentiation through their direct contact with erythroblasts via an EPO-independent mechanism, with cultured erythroblasts proliferating threefold more when in contact with macrophages [41,42].

Studies employing reconstituted EBIs have been used to elucidate proteins involved in erythroblast and macrophage interactions. Erythroblast–macrophage attachments within the EBI niche are important in promoting erythropoiesis, and the adhesion proteins that facilitate these attachments are critical for island integrity. One of the first proteins identified to be involved in erythroblast–macrophage attachment was the $\alpha4\beta1$ integrin on erythroblasts that binds VCAM-1 on macrophages [55]. Blocking monoclonal antibodies against $\alpha4\beta1$ integrin and VCAM-1 significantly impaired erythroblast–macrophage attachment, indicating that this interaction is critical for island integrity [55]. Reconstitution of EBIs in vitro has been a powerful model in which to observe the dynamic relationship between cell types, but the strategy is limited by culture conditions and does not allow for investigation of the possible interactions of other cell types in the bone marrow. Furthermore, it is unclear how this relates to the in vivo situation and how transferable it would be to the human EBI (Table 1).

Macrophage depletion models

Macrophage depletion models in stress erythropoiesis. Macrophage depletion has been a particularly useful model in which to investigate the role of the macrophage compartment of the EBI niche in vivo. Macrophages can be depleted by administering clodronate-encapsulated liposomes, which are phagocytosed and induce apoptosis [61–63], or by using the CD169-DTR mouse strain, which expresses the human diphtheria toxin receptor (DTR) under the control of the endogenous *Siglec-1* (CD169) promoter [64]. Macrophage depletion is relatively easy to achieve, and can be applied to the different genetic models (Table 1).

Macrophage depletion models have been used to study the contribution of EBI macrophages to stress erythropoiesis in addition to the contribution of microenvironmental cells; for example, splenic endothelial cells secrete stem cell factor (SCF) that mediates stress erythropoiesis in response to myeloablation, bleeding, and pregnancy [65]. Macrophage depletion has been found to severely compromise stress erythropoiesis, impairing recovery from anemia, acute blood loss, and myeloablation. In a study in which macrophages were depleted using clodronate, there was a significant reduction in reticulocytes and erythroid precursors in both the bone marrow (BM) and spleen [62,63]. Depletion of CD169$^+$ macrophages using the CD169-DTR mouse model impaired recovery from hemolytic anemia and acute blood loss, and this was associated with a reduced number of EBIs and erythroblasts in bone marrow [66]. In two models of acute RBC reduction, phenylhydrazine-induced anemia and acute blood loss, a delay in hematocrit recovery was observed. There was also a delay in the recovery of erythroblast numbers in

myeloablation following BM transplant and after challenge with the myeloablative agent 5-fluorouracil [66].

One hallmark of stress erythropoiesis is the shift from erythropoiesis in the BM to extramedullary sites such as the liver and spleen and the generation of stress erythroid progenitors (SEPs) [4,67–69]. During recovery from anemic stress, CCL2 production recruits monocytes to the spleen, where they associate with SEPs and differentiate into red pulp macrophages (RPMs) creating new EBIs [70]. Under steady-state conditions, RPMs, a population of tissue-resident macrophages, contribute to maintaining erythroid homeostasis by phagocytosing senescent erythrocytes, recycling iron and degrading heme [71]. These RPMs have also been observed to form EBIs after transplantation and myeloablation [72]. Splenic RPMs secrete BMP4, which is essential for the recovery of erythroid cells after a bone marrow transplant-induced model of myeloablation [66]. Spleens of $CD169^+$ macrophage-depleted mice, which had impaired erythroblast recovery, expressed significantly less BMP4, and reciprocal transplantation studies identified splenic RPM as the source of this BMP4 [66].

Macrophage depletion models in disease. Macrophage depletion models have been used to implicate the macrophage compartment of the EBI niche in various RBC diseases. In a murine model of polycythemia vera (PCV), a disease in which there is excessive production of erythroid cells as a result of a point mutation in JAK2 ($JAK2^{V617F}$), blood hematocrit was normalized following depletion of $CD169^+$ macrophages [66,73,74]. Using the $Jak2^{V617F/+}$ murine model of PCV, researchers found that clodronate-mediated macrophage depletion also normalized RBC numbers [63,75]. JAK2 is a component of the EpoR signaling cascade, and the results of these macrophage depletion studies imply that the hyperactive EpoR signaling caused by the $JAK2^{V617F}$ mutant protein within the macrophage compartment contributes to the disease phenotype in this model of PCV [74]. The fact that the number of macrophages increases in response to EPO administration in control mice confirms that EpoR signaling is indeed active in macrophages as well as erythroid cells within the EBI [76], and this is further supported by the expansion of both erythrocytes and macrophages in mouse models of erythrocytosis [76].

Macrophage depletion also improved the phenotype of a mouse model of β-thalassemia, with mice treated with clodronate exhibiting increased hemoglobin and RBC numbers [63,77]. Therefore, macrophages have been found to influence disease phenotypes by both increasing and decreasing RBC numbers.

Although macrophage depletion has been an effective method in elucidating the role of EBI macrophages in stress and disease erythropoiesis, having been demonstrated to deplete $CD169^+$ $VCAM^+$ EBI macrophages, EBI macrophages are not exclusively depleted (Table 1). It is unclear what contribution, if any, the depletion of other macrophage subsets has on erythropoiesis. Further studies to isolate and deplete only EBI macrophages are needed to fully elucidate their contribution to disease.

Genetically modified mouse models

With the advent of gene editing technology, the role of specific genes in the EBI niche could be assessed in vivo using genetically modified mice (Table 1). Targeted deletion of genes hypothesized to have an important role in EBIs was used to strengthen observations made in previous in vitro studies. For example, antibody inhibition studies in vitro identified that ICAM-4, a member of the intercellular adhesion molecule family expressed in erythroid cells, binds to $\alpha4\beta1$integrin and αv integrins [78]. The subsequent production of ICAM-4-null mice confirmed that its interaction with αv integrins on macrophages is involved in maintaining EBI integrity [58]. Islands were harvested intact from ICAM-4-null mice or reconstituted in vitro, and a significant decrease in reconstituted islands was observed in isolates from ICAM-4-null compared with WT mice, supporting the functional role of αv integrin in EBI macrophages [58].

Targeted gene inactivation using a gene trapping approach confirmed the in vivo function of Emp (erythroblast–macrophage protein), which had been previously implicated as an important mediator of erythroblast–macrophage attachment in vitro [41,79]. Emp-null embryos die shortly after birth and present with an increased number of nucleated, immature erythrocytes in their peripheral blood [80]. The phenotype is especially stark in the fetal liver, where almost no EBIs were observed. Interestingly, control wild-type macrophages were still able to bind Emp-deficient erythroblasts, but these erythroblasts failed to enucleate. Further insight into macrophage/erythroid interactions via Emp was gleaned from a macrophage-specific conditional gene deletion of *Emp (Maea)* that resulted in severely impaired EBI formation, whereas deletion in the erythroid lineage had no detrimental effect [81]. Together, these studies indicate that Emp regulates the maintenance of macrophages and that Emp-mediated adhesion to erythroblasts must involve another currently unidentified receptor on erythroid cells [81].

Gene targeting studies in mice have also addressed the role of EBI macrophages in promoting enucleation via cell–cell attachments and in its function to phagocytose nuclei extruded from the erythroblasts. Nuclei are extruded as “pyrenocytes,” small, nucleated cells with a cytoplasmic ring [9]. Pyrenocytes externalize

phosphatidylserine, providing a signal to macrophages for engulfment [47]. The endonuclease DNaseII (Dnase2) in EBI macrophages destroys the nucleus expelled from erythroblasts, as demonstrated in DNaseII-deficient mice, where undegraded DNA stimulates EBI macrophages to express interferon (IFN)−β, therefore inhibiting erythropoiesis [82]. It was particularly interesting to note that expression of *Dnase2a* in EBI macrophages is regulated by KLF1 [7,83], a transcription factor that was first identified as a master regulator within the erythroid lineage, with an important role in regulating the later stages of RBC production, including globin switching to the activation of erythroid-specific genes [84–87]. Adding to its critical intrinsic role, an extrinsic role for KLF1 in the macrophage compartment of the erythroblastic island niche was first implicated using a KLF1-eGFP reporter mouse strain where GFP was detected in EBI macrophages and genes associated with island integrity such as VCAM1 were expressed at a higher level in KLF1-GFP$^+$ macrophages [8,88,89].

Stress erythropoiesis has been reported to be particularly sensitive to modifications in the EBI niche, and mouse genetic models have also been used to assess the role of specific genes in that process [4]. Steady-state erythropoiesis and stress erythropoiesis are thought to be regulated by different mechanisms, with EBI macrophages being more strongly implicated under stress conditions. Growth differentiation factor 15 (Gdf15), for example, is an essential regulator of stress, but not steady-state, erythropoiesis in both mice and humans [90,91]. *Gdf15*$^{-/-}$ mice exhibit a reduced expansion of the splenic stress erythroid niche from monocyte recruitment, resulting in an impaired proliferation of stress erythroid progenitors (SEPs) [91]. It was reported that Gdf15 signaling modulates Hif2α-dependent expression of BMP4 in macrophages through Vhl inhibition, which, in turn, regulates SEP proliferation [91].

Characterization of the Epor-eGFP knockin reporter mouse revealed that the majority of EBI macrophages express the Epo receptor, implying that Epo can act on both macrophages and erythroid cells [56]. It was recently found that Epo/Stat signaling in splenic EBI macrophages represses the Wnt signaling that promotes SEP proliferation so the end result of macrophage Epo signaling is the differentiation of SEPs into functionally mature RBCs [92].

Stress erythropoiesis can also be induced during inflammation, where bone marrow hematopoiesis favors the production of innate immune effector cells at the expense of RBC production [93]. Inflammation-induced anemia is common in patients with chronic inflammation, and several pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and IFN-γ, have been reported to inhibit steady-state erythropoiesis [94–97]. A recent study using a mouse model of sterile inflammation revealed this inhibition is compensated by an increase in stress erythropoiesis. Signaling through Toll-like receptors (TLRs) stimulated the phagocytosis of erythrocytes by splenic macrophages, facilitating heme-dependent expression of the transcription factor SPI-C, which, in turn, promoted the expression of both *Gdf15* and *Bmp4,* which act to increase the proliferation of SEPs [98].

Could changes in the EBI niche contribute to RBC disorders as well as stress erythropoiesis, and if so, could modulation of the EBI niche be exploited to treat these RBC conditions? Experimental evidence in mouse models suggests that modulation of the EBI niche could be effective in treating RBC disorders. EPO, a hormone widely used to stimulate erythroblast production and maturation, is not always effective, with many anemic patients unresponsive to treatment [99,100]. Growth arrest-specific factor 6 (Gas6), a protein secreted by erythroblasts in response to EPO, enhances EPO signaling directly by activating the Akt survival pathway in erythroblasts and indirectly by reducing the release of erythroid-inhibitory factors from macrophages in the EBI niche [101]. In a *Gas6*$^{-/-}$ mouse model, there is an increase in the expression of various cytokines known to inhibit erythropoiesis, for example, interleukin (IL)-10, IL-13, and TNF-α [101]. Thus, Gas6 could be a targeted as a potential therapeutic option that would act on both erythroblasts and EBI macrophages to promote erythropoiesis.

One major advantage of using genetic models is that, unlike in vitro models of the EBI niche, genetic models in mice have allowed for the dissection of the role of specific genes in vivo. However, they are much more expensive and complex than in vitro studies and still may not accurately reflect the human system (Table 1).

Modeling the human EBI niche

The mouse models described have all contributed significantly to our understanding but how closely this resembles the human EBI niche is unclear. Work so far has focused on the EBI macrophage, a key cell type in promoting erythropoiesis, but this has not allowed for investigation into the involvement of other cell types (Table 1). Human EBIs can be isolated from bone specimens resected during surgery, for example, ribs during thoracic surgery, or from sectioned bone marrow [45]. Sourcing this tissue, however, is extremely difficult. Thus, the majority of studies into the human EBI niche have relied on in vitro modeling, mainly using cells sourced from peripheral blood. Recently, components of the EBI niche have been derived from either pluripotent stem cells or CD34$^+$ hematopoietic

progenitors and used to further characterize the human EBI niche [8].

Hematopoietic progenitor cell-derived macrophages

Modeling of the human EBI niche has been possible using $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) that can differentiate into macrophages and erythroid cells. An Mpl-based cell growth switch system was reported to drive macrophage-associated erythropoiesis, and the macrophages produced in this system developed a phenotype similar to that of EBI macrophages; they expressed EMP, ICAM-4, CD163, and DNASE2 and supported the maturation of erythroid cells to the orthochromatic stage [102,103].

The addition of the synthetic glucocorticoid dexamethasone to differentiating $CD34^+$ HPCs has been particularly useful in modeling human stress erythropoiesis in vitro [104]. As erythroid differentiation is inhibited by dexamethasone, the consequential expansion of pro-erythroblasts mimics the stressed situation. Interestingly the small population of macrophages that are generated under these conditions (3% of total culture) interact with the expanding erythroblasts to form EBIs. The presence of dexamethasone promoted the maturation of $CD169^+$ macrophages [104], the cell phenotype that is known to promote erythropoiesis under stress conditions in murine models [66]. Taken together, evidence suggests that the EBI macrophages involved in stress erythropoiesis are likely to be distinct from those in the steady state.

Monocyte-derived macrophages

Peripheral blood mononuclear cells (PBMCs) have been used to illustrate that macrophages can function as a ferritin iron source for cultured human erythroblasts to synthesize hemoglobin, a long-suspected role of EBI macrophages [44]. In vitro modeling of the human EBI niche has been consistent with findings obtained in the murine system indicating that contact with macrophages promotes erythroblast proliferation. Co-culture of $CD14^+$ PBMC-derived intermediate monocytes/macrophages with $CD34^+$ progenitors enhanced erythropoiesis by supporting progenitor cell survival [105], and the addition of glucocorticoids induced the differentiation of monocytes to macrophages with an EBI-macrophage phenotype [106].

Not only does this modeling rely on the availability of monocyte-derived macrophages, it is important to note that different effects have been reported on maturation and enucleation [63,105]. Furthermore, monocyte-derived macrophages may not accurately reflect the developmental ontogeny of EBI macrophages [107–110]. To improve modeling of the human EBI in vitro, macrophages were derived from induced pluripotent stem cells (iPSCs) that can provide a limitless resource. iPSC-derived macrophages can be harvested repeatedly from cultures, which greatly increases the yield of available macrophages compared with those derived from monocytes [109,111]. Furthermore, iPSC-derived macrophages have been found to share ontogeny with *MYB*-independent tissue-resident macrophages, and although the exact developmental origins of EBI macrophages have not been determined, it is thought that they arise from yolk sac-derived EMPs, therefore sharing ontogeny with tissue-resident macrophages [108].

iPSC-derived macrophages

iPSC-derived macrophages were reported to promote the maturation and enucleation of RBCs differentiating from $CD34^+$ hematopoietic progenitor cells in an in vitro model of the human EBI niche [8]. A tamoxifen-ER^{T2} expression system was used whereby the transcription factor KLF1 was expressed under the control of the constitutive CAG promoter and translocated to the nucleus on addition of tamoxifen. Nuclear translocation and, thus, activation of KLF1 during the differentiation of iPSC-derived macrophages led to the production of macrophages with an "EBI macrophage"-like phenotype. Macrophages expressing higher levels of KLF1 were better able to promote erythroblast maturation, resulting in an increase in enucleated RBCs in the culture [8]. The use of this KLF1 expression system therefore supported previous findings of an extrinsic role for KLF1 in EBI macrophages [8,89].

The use of this unique in vitro model of the human EBI enabled the identification of KLF1-regulated genes encoding secreted factors, including IL-33, SERPINB2, and ANGPLT7, that were shown to be important for promoting erythropoiesis [8]. Addition of all three of these cytokines to differentiating $CD34^+$ hematopoietic stem and progenitor cell cultures significantly increased the absolute number of mature, enucleated cells, with removal of individual cytokines resulting in an overall reduction in the number of mature enucleated cells. Removal of IL-33 resulted in the most significant reduction in mature cells, but IL-33 alone did not improve maturation, implying that it acts in synergy with the other factors [8]. This study found that in vitro modeling of the EBI niche using iPSC-derived macrophages provides a powerful tool to identify and characterize novel factors and the key signaling and regulatory pathways involved in erythropoiesis. It will be possible to use this in vitro model in the future to assess the effects of these novel factors as therapeutic options for patients with RBC disorders. For example, cytokines such as IL-33 could be investigated for their ability to promote RBC proliferation and maturation in anemic patients who do not respond to EPO treatment, and small molecules to activate or block key signaling

pathways could be tested as potential therapies [99,100].

The ability to genetically engineer iPSCs and, thus, differentiated macrophages represents a promising approach to dissecting the role of individual genes within the human EBI niche [112,113]. The wide array of genetic tools also allows for controlled temporal activation and knockout of specific genetic pathways. The tamoxifen-ER^{T2} is particularly useful as it appears to recapitulate endogenous gene expression levels, avoiding very high, nonphysiological expression levels often seen in standard transgenic or viral expression systems [8]. The human iPSC-derived macrophage strategy also holds great potential in the context of RBC disorders, in which pathogenic gene mutations can be introduced into EBI-like macrophages to investigate whether genetic deficiencies in niche contribute to disease pathology.

In vitro modeling of the human EBI could also be used for patient-specific drug testing. This is especially useful for diseases in which animal models do not exactly recapitulate the disease. For example, the neonatal anemia *nan* mouse carries a mutation (KLF1-E339D) homologous to the KLF1-E325K mutation observed in patients with type IV congenital dyserythropoietic anemia (CDA), but the phenotype is quite different [114,115]. Macrophages and RBCs differentiated from patient-derived iPSCs would allow for the in vitro modeling of EBIs in disease and could prove especially useful for dissecting the contributions of each cell type to disease pathology. By use of an iPSC line derived from a CDA patient, it was shown that the KLF1-E325K mutation induces cell cycle arrest in differentiated erythroid cells [116]. This was comparable to data derived from erythroid cells differentiated from patient-derived CD34+ progenitors where RNA sequencing revealed the dysregulation of cell cycle genes [117]. In the future, the production of macrophages from this patient-derived iPSC line will elucidate whether the presence of the KLF1-E325K mutant protein in the EBI niche contributes to the pathology of the disease and will determine whether targeting the niche might be a therapeutic option.

Conclusions

The concept of the EBI has greatly progressed since it was first described by Bessis in 1958 [1] with central macrophage functions, such as the role of cell contact in promoting erythropoiesis, now well defined. In vitro models of the human and murine EBI, both intact and reconstituted, are relatively cheap and simple systems that have allowed for visual examination of the niche. Their simplicity, however, does not allow for dissection of the roles of individual genes, and in the human system, sourcing primary tissue can be difficult (Table 1). Although not possible in the human system, macrophage depletion and genetic models have been incredibly useful for understanding the role of individual genes within the mouse EBI niche. These genes are now beginning to be investigated in the human EBI niche, in which modeling using iPSC-derived macrophages now allows for the kinds of genetic manipulation employed to study the mouse system (Table 1).

The advances that have been made in modeling murine and human EBIs both in vivo and in vitro have great potential to be utilized to further examine the role of the EBI niche during stress erythropoiesis and in RBC disorders, especially by elucidating molecular mechanisms that could be targeted in their treatment. To recreate the EBI niche during stress erythropoiesis it will be interesting to assess whether iPSC-derived macrophages exhibit the characteristics of splenic RPMs and, if not, to devise culture conditions or genetic strategies to mimic their phenotype. The knowledge gained from the various models of the EBI niche could also aid in research to improve the production of functional RBCs from iPSC in vitro for clinical use. Blood transfusion, the most common treatment for RBC disorders, is reliant on donor supply and compatibility and has side effects such as iron overload in patients who require regular transfusions [118]. Therefore, there is worldwide interest in the development of a donor-free supply of RBCs from a renewable source such as iPSCs. Although significant progress has been made in this area, the strategy has not been successful in providing a stable source of enucleated RBCs that can be scaled up for therapeutic uses [119–123].

Acknowledgments

Work in the author's laboratory is funded by the Medical Research Council (LMF) and the Wellcome Trust (AM). We thank Niamh B. McNamara for help with the graphics and Shyam Sushama and Antonella Fidanza for critical comments on the article. Figure 1 and the graphical abstract were created with BioRender.com.

References

1. Bessis M. [Erythroblastic island, functional unity of bone marrow]. Rev Hematol. 1958;13:8–11.
2. Mohandas N, Prenant M. Three-dimensional model of bone marrow. Blood. 1978;51:633–643.
3. Sonoda Y, Sasaki K. Hepatic extramedullary hematopoiesis and macrophages in the adult mouse: histometrical and immunohistochemical studies. Cells Tissues Organs. 2012;196:555–564.
4. Paulson RF, Hariharan S, Little J. Stress erythropoiesis: definitions and models for its study. Exp Hematol.. Online 2 August 2020. https://doi.org/10.1016/j.exphem.2020.07.011.
5. Berman I. The ultrastructure of erythroblastic islands and reticular cells in mouse bone marrow. J Ultrastruct Res. 1967;17:291–313.

6. Bessis MC, Breton-Gorius J. Iron metabolism in the bone marrow as seen by electron microscopy: a critical review. Blood. 1962;19:635–663.
7. Porcu S, Manchinu MF, Marongiu MF, et al. Klf1 affects DNase II-alpha expression in the central macrophage of a fetal liver erythroblastic island: a non-cell-autonomous role in definitive erythropoiesis. Mol Cell Biol. 2011;31:4144–4154.
8. Lopez-Yrigoyen M, Yang CT, Fidanza A, et al. Genetic programming of macrophages generates an in vitro model for the human erythroid island niche. Nat Commun. 2019;10:881.
9. McGrath KE, Kingsley PD, Koniski AD, Porter RL, Bushnell TP, Palis J. Enucleation of primitive erythroid cells generates a transient population of ''pyrenocytes'' in the mammalian fetus. Blood. 2008;111:2409–2417.
10. Policard A, Bessis M. Micropinocytosis and rhopheocytosis. Nature. 1962;194:110–111.
11. Lo Celso C, Fleming HE, Wu JW, et al. Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. Nature. 2009;457:92–96.
12. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006;25:977–988.
13. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005;121:1109–1121.
14. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014;505:327–334.
15. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? Int J Lab Hematol. 2016;38(Suppl 1):123–132.
16. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. Public Health Nutr. 2009;12:444–454.
17. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;123:615–624.
18. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2019 update on diagnosis, risk-stratification and management. Am J Hematol.. 2019;94:133–143.
19. Beutler E. Genetic disorders of human red blood cells. JAMA. 1975;233:1184–1188.
20. Socie G, Mary JY, de Gramont A, et al. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology. Lancet.. 1996;348:573–577.
21. Muncie HL Jr, Campbell J. Alpha and beta thalassemia. Am Fam Physician. 2009;80:339–344.
22. Williams TN, Thein SL. Sickle cell anemia and its phenotypes. Annu Rev Genom Hum Genet. 2018;19:113–147.
23. Fibach E, Rachmilewitz EA. Pathophysiology and treatment of patients with beta-thalassemia—an update. F1000Res. 2017;6:2156.
24. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of iron deficiency anemia. Gastroenterol Hepatol (NY). 2015;11:241–250.
25. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin: results of a combined phase I and II clinical trial. N Engl J Med. 1987;316:73–78.
26. Debeljak N, Sytkowski AJ. Erythropoietin and erythropoiesis stimulating agents. Drug Test Anal. 2012;4:805–812.
27. Glaspy J, Bukowski R, Steinberg D, Taylor C, Tchekmedyian S, Vadhan-Raj S. Impact of therapy with epoetin alfa on clinical outcomes in patients with nonmyeloid malignancies during cancer chemotherapy in community oncology practice. Procrit Study Group. J Clin Oncol.. 1997;15:1218–1234.
28. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006;355:1233–1243.
29. Debureaux P-E, Cacace F, Silva BGP, et al. Hematological response to eculizumab in paroxysmal nocturnal hemoglobinuria: application of a novel classification to identify unmet clinical needs and future clinical goals. Blood. 2019;134(Suppl 1):3517.
30. Saso R, Marsh J, Cevreska L, et al. Bone marrow transplants for paroxysmal nocturnal haemoglobinuria. Br J Haematol. 1999;104:392–396.
31. Locasciulli A, Oneto R, Bacigalupo A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). Haematologica. 2007;92:11–18.
32. Mohty M, Apperley JF. Long-term physiological side effects after allogeneic bone marrow transplantation. Hematology Am Soc Hematol Educ Program. 2010;2010:229–236.
33. Edvardsson L, Dykes J, Olofsson T. Isolation and characterization of human myeloid progenitor populations—TpoR as discriminator between common myeloid and megakaryocyte/erythroid progenitors. Exp Hematol. 2006;34:599–609.
34. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature. 2000;404:193–197.
35. Gregory CJ, Eaves AC. Three stages of erythropoietic progenitor cell differentiation distinguished by a number of physical and biologic properties. Blood. 1978;51:527–537.
36. Gregory CJ, Eaves AC. Human marrow cells capable of erythropoietic differentiation in vitro: definition of three erythroid colony responses. Blood. 1977;49:855–864.
37. Granick S, Levere RD. Heme synthesis in erythroid cells. Prog Hematol. 1964;4:1–47.
38. Yeo JH, Lam YW, Fraser ST. Cellular dynamics of mammalian red blood cell production in the erythroblastic island niche. Biophys Rev. 2019;11:873–894.
39. Chasis JA, Prenant M, Leung A, Mohandas N. Membrane assembly and remodeling during reticulocyte maturation. Blood. 1989;74:1112–1120.
40. Chen K, Liu J, Heck S, Chasis JA, An X, Mohandas N. Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. Proc Natl Acad Sci USA. 2009;106:17413–17418.
41. Hanspal M, Hanspal JS. The association of erythroblasts with macrophages promotes erythroid proliferation and maturation: a 30-kD heparin-binding protein is involved in this contact. Blood. 1994;84:3494–3504.
42. Rhodes MM, Kopsombut P, Bondurant MC, Price JO, Koury MJ. Adherence to macrophages in erythroblastic islands enhances erythroblast proliferation and increases erythrocyte production by a different mechanism than erythropoietin. Blood. 2008;111:1700–1708.
43. Allen TD, Dexter TM. Ultrastructural aspects of erythropoietic differentiation in long-term bone marrow culture. Differentiation. 1982;21:86–94.
44. Leimberg MJ, Prus E, Konijn AM, Fibach E. Macrophages function as a ferritin iron source for cultured human erythroid precursors. J Cell Biochem. 2008;103:1211–1218.
45. Lee SH, Crocker PR, Westaby S, et al. Isolation and immunocytochemical characterization of human bone marrow stromal

macrophages in hemopoietic clusters. J Exp Med. 1988;168: 1193–1198.
46. Yokoyama T, Kitagawa H, Takeuchi T, Tsukahara S, Kannan Y. No apoptotic cell death of erythroid cells of erythroblastic islands in bone marrow of healthy rats. J Vet Med Sci. 2002;64:913–919.
47. Yoshida H, Kawane K, Koike M, Mori Y, Uchiyama Y, Nagata S. Phosphatidylserine-dependent engulfment by macrophages of nuclei from erythroid precursor cells. Nature. 2005;437:754–758.
48. Ganzoni A, Hillman RS, Finch CA. Maturation of the macroreticulocyte. Br J Haematol. 1969;16:119–135.
49. Gronowicz G, Swift H, Steck TL. Maturation of the reticulocyte in vitro. J Cell Sci. 1984;71:177–197.
50. Le Charpentier Y, Prenant M. [Isolation of erythroblastic islands. Study by optical and scanning electron microscopy (author's translation)]. Nouv Rev Fr Hematol.. 1975;15:119–140.
51. Hume DA, Robinson AP, MacPherson GG, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: relationship between macrophages, Langerhans cells, reticular cells, and dendritic cells in lymphoid and hematopoietic organs. J Exp Med. 1983;158:1522–1536.
52. Yokoyama T, Etoh T, Kitagawa H, Tsukahara S, Kannan Y. Migration of erythroblastic islands toward the sinusoid as erythroid maturation proceeds in rat bone marrow. J Vet Med Sci. 2003;65:449–452.
53. Crocker PR, Gordon S. Isolation and characterization of resident stromal macrophages and hematopoietic cell clusters from mouse bone marrow. J Exp Med. 1985;162:993–1014.
54. Seu KG, Papoin J, Fessler R, et al. Unraveling macrophage heterogeneity in erythroblastic islands. Front Immunol. 2017;8:1140.
55. Sadahira Y, Yoshino T, Monobe Y. Very late activation antigen 4-vascular cell adhesion molecule 1 interaction is involved in the formation of erythroblastic islands. J Exp Med. 1995; 181:411–415.
56. Li W, Wang Y, Zhao H, et al. Identification and transcriptome analysis of erythroblastic island macrophages. Blood. 2019; 134:480–491.
57. Breton-Gorius J, Guichard J, Vainchenker W. Absence of erythroblastic islands in plasma clot culture and their possible reconstitution after clot lysis. Blood Cells. 1979;5:461–469.
58. Lee G, Lo A, Short SA, et al. Targeted gene deletion demonstrates that the cell adhesion molecule ICAM-4 is critical for erythroblastic island formation. Blood. 2006;108:2064–2071.
59. Liu XS, Li XH, Wang Y, et al. Disruption of palladin leads to defects in definitive erythropoiesis by interfering with erythroblastic island formation in mouse fetal liver. Blood. 2007; 110:870–876.
60. Wang L, Yu H, Cheng H, et al. Deletion of Stk40 impairs definitive erythropoiesis in the mouse fetal liver. Cell Death Dis. 2017;8(3):e2722.
61. Weisser SB, van Rooijen N, Sly LM. Depletion and reconstitution of macrophages in mice. J Vis Exp. 2012;66:4105.
62. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J Immunol Methods. 1994;174:83–93.
63. Ramos P, Casu C, Gardenghi S, et al. Macrophages support pathological erythropoiesis in polycythemia vera and beta-thalassemia. Nat Med. 2013;19:437–445.
64. Miyake Y, Asano K, Kaise H, Uemura M, Nakayama M, Tanaka M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J Clin Invest. 2007;117:2268–2278.
65. Inra CN, Zhou BO, Acar M, et al. A perisinusoidal niche for extramedullary haematopoiesis in the spleen. Nature. 2015; 527:466–471.
66. Chow A, Huggins M, Ahmed J, et al. CD169(+) macrophages provide a niche promoting erythropoiesis under homeostasis and stress. Nat Med. 2013;19:429–436.
67. Harandi OF, Hedge S, Wu DC, McKeone D, Paulson RF. Murine erythroid short-term radioprotection requires a BMP4-dependent, self-renewing population of stress erythroid progenitors. J Clin Invest. 2010;120:4507–4519.
68. Lenox LE, Perry JM, Paulson RF. Extramedullary erythropoiesis in the adult liver requires BMP-4/Smad5-dependent signaling. Blood. 2005;105:2741–2748.
69. Porayette P, Paulson RF. BMP4/Smad5 dependent stress erythropoiesis is required for the expansion of erythroid progenitors during fetal development. Dev Biol. 2008;317:24–35.
70. Liao C, Prabhu KS, Paulson RF. Monocyte-derived macrophages expand the murine stress erythropoietic niche during the recovery from anemia. Blood. 2018;132:2580–2593.
71. Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. Cell. 2004;117:285–297.
72. Sadahira Y, Mori M, Kimoto T. Participation of radioresistant Forssman antigen-bearing macrophages in the formation of stromal elements of erythroid spleen colonies. Br J Haematol. 1989;71:469–474.
73. Zhan H, Spivak JL. The diagnosis and management of polycythemia vera, essential thrombocythemia, and primary myelofibrosis in the JAK2 V617F era. Clin Adv Hematol Oncol. 2009;7:334–342.
74. Zhao R, Xing S, Li Z, et al. Identification of an acquired JAK2 mutation in polycythemia vera. J Biol Chem. 2005;280:22788–22792.
75. Mullally A, Lane SW, Ball B, et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. Cancer Cell. 2010;17:584–596.
76. Wang J, Hayashi Y, Yokota A, et al. Expansion of EPOR-negative macrophages besides erythroblasts by elevated EPOR signaling in erythrocytosis mouse models. Haematologica. 2018; 103:40–50.
77. Ciavatta DJ, Ryan TM, Farmer SC, Townes TM. Mouse model of human beta zero thalassemia: targeted deletion of the mouse beta maj- and beta min-globin genes in embryonic stem cells. Proc Natl Acad Sci USA. 1995;92:9259–9263.
78. Spring FA, Parsons SF, Ortlepp S, et al. Intercellular adhesion molecule-4 binds alpha(4)beta(1) and alpha(V)-family integrins through novel integrin-binding mechanisms. Blood. 2001;98: 458–466.
79. Hanspal M, Smockova Y, Uong Q. Molecular identification and functional characterization of a novel protein that mediates the attachment of erythroblasts to macrophages. Blood. 1998;92: 2940–2950.
80. Soni S, Bala S, Gwynn B, Sahr KE, Peters LL, Hanspal M. Absence of erythroblast macrophage protein (Emp) leads to failure of erythroblast nuclear extrusion. J Biol Chem. 2006; 281:20181–20189.
81. Wei Q, Boulais PE, Zhang D, Pinho S, Tanaka M, Frenette PS. Maea expressed by macrophages, but not erythroblasts, maintains postnatal murine bone marrow erythroblastic islands. Blood. 2019;133:1222–1232.
82. Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S. Lethal anemia caused by interferon-beta produced in mouse embryos carrying undigested DNA. Nat Immunol. 2005;6:49–56.

83. Kawane K, Fukuyama H, Kondoh G, et al. Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver. Science. 2001;292:1546–1549.
84. Miller IJ, Bieker JJ. A novel, erythroid cell-specific murine transcription factor that binds to the CACCC element and is related to the Kruppel family of nuclear proteins. Mol Cell Biol. 1993;13:2776–2786.
85. Funnell AP, Maloney CA, Thompson LJ, et al. Erythroid Kruppel-like factor directly activates the basic Kruppel-like factor gene in erythroid cells. Mol Cell Biol. 2007;27:2777–2790.
86. Chen X, Bieker JJ. Erythroid Kruppel-like factor (EKLF) contains a multifunctional transcriptional activation domain important for inter- and intramolecular interactions. EMBO J. 1996;15:5888–5896.
87. Tallack MR, Whitington T, Yuen WS, et al. A global role for KLF1 in erythropoiesis revealed by ChIP-seq in primary erythroid cells. Genome Res. 2010;20:1052–1063.
88. Siatecka M, Bieker JJ. The multifunctional role of EKLF/KLF1 during erythropoiesis. Blood. 2011;118:2044–2054.
89. Xue L, Galdass M, Gnanapragasam MN, Manwani D, Bieker JJ. Extrinsic and intrinsic control by EKLF (KLF1) within a specialized erythroid niche. Development. 2014;141:2245–2254.
90. Xiang J, Wu DC, Chen Y, Paulson RF. In vitro culture of stress erythroid progenitors identifies distinct progenitor populations and analogous human progenitors. Blood. 2015;125:1803–1812.
91. Hao S, Xiang J, Wu DC, et al. Gdf15 regulates murine stress erythroid progenitor proliferation and the development of the stress erythropoiesis niche. Blood Adv. 2019;3:2205–2217.
92. Chen Y, Xiang J, Qian F, et al. Epo receptor signaling in macrophages alters the splenic niche to promote erythroid differentiation. Blood. 2020;136:235–246.
93. Jurado RL. Iron, infections, and anemia of inflammation. Clin Infect Dis. 1997;25:888–895.
94. Nemeth E, Ganz T. Anemia of inflammation. Hematol Oncol Clin North Am. 2014;28:671–681. vi.
95. Libregts SF, Gutiérrez L, de Bruin AM, et al. Chronic IFN-γ production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. Blood. 2011;118:2578–2588.
96. Rusten LS, Jacobsen SE. Tumor necrosis factor (TNF)-alpha directly inhibits human erythropoiesis in vitro: role of p55 and p75 TNF receptors. Blood. 1995;85:989–996.
97. Schooley JC, Kullgren B, Allison AC. Inhibition by interleukin-1 of the action of erythropoietin on erythroid precursors and its possible role in the pathogenesis of hypoplastic anaemias. Br J Haematol. 1987;67:11–17.
98. Bennett LF, Liao C, Quickel MD, et al. Inflammation induces stress erythropoiesis through heme-dependent activation of SPI-C. Sci Signal. 2019;12(598). eeap7336.
99. Richmond TD, Chohan M, Barber DL. Turning cells red: signal transduction mediated by erythropoietin. Trends Cell Biol. 2005;15:146–155.
100. Lacombe C. Resistance to erythropoietin. N Engl J Med. 1996; 334:660–662.
101. Angelillo-Scherrer A, Burnier L, Lambrechts D, et al. Role of Gas6 in erythropoiesis and anemia in mice. J Clin Invest. 2008;118:583–596.
102. Belay E, Hayes BJ, Blau CA, Torok-Storb B. Human cord blood and bone marrow CD34+ cells generate macrophages that support erythroid islands. PLoS One. 2017;12:e0171096.
103. Belay E, Miller CP, Kortum AN, Torok-Storb B, Blau CA, Emery DW. A hyperactive Mpl-based cell growth switch drives macrophage-associated erythropoiesis through an erythroid −megakaryocytic precursor. Blood. 2015;125:1025–1033.
104. Falchi M, Varricchio L, Martelli F, et al. Dexamethasone targeted directly to macrophages induces macrophage niches that promote erythroid expansion. Haematologica. 2015;100:178–187.
105. Heideveld E, Masiello F, Marra M, et al. CD14+ cells from peripheral blood positively regulate hematopoietic stem and progenitor cell survival resulting in increased erythroid yield. Haematologica. 2015;100:1396–1406.
106. Heideveld E, Hampton-O'Neil LA, Cross SJ, et al. Glucocorticoids induce differentiation of monocytes towards macrophages that share functional and phenotypical aspects with erythroblastic island macrophages. Haematologica. 2018;103:395–405.
107. Alasoo K, Martinez FO, Hale C, et al. Transcriptional profiling of macrophages derived from monocytes and iPS cells identifies a conserved response to LPS and novel alternative transcription. Sci Rep. 2015;5:12524.
108. Buchrieser J, James W, Moore MD. Human induced pluripotent stem cell-derived macrophages share ontogeny with MYB-independent tissue-resident macrophages. Stem Cell Rep. 2017; 8:334–345.
109. Haideri SS, McKinnon AC, Taylor AH, et al. Injection of embryonic stem cell derived macrophages ameliorates fibrosis in a murine model of liver injury. NPJ Regen Med. 2017;2:14.
110. Heideveld E, van den Akker E. Digesting the role of bone marrow macrophages on hematopoiesis. Immunobiology. 2017;222: 814–822.
111. Lopez-Yrigoyen M, Fidanza A, Cassetta L, et al. A human iPSC line capable of differentiating into functional macrophages expressing ZsGreen: a tool for the study and in vivo tracking of therapeutic cells. Philos Trans R Soc Lond B Biol Sci. 2018;373(1750):20170219.
112. Hockemeyer D, Jaenisch R. Induced pluripotent stem cells meet genome editing. Cell Stem Cell. 2016;18:573–586.
113. Chang CY, Ting HC, Su HL, Jeng JR. Combining induced pluripotent stem cells and genome editing technologies for clinical applications. Cell Transplant. 2018;27:379–392.
114. Siatecka M, Sahr KE, Andersen SG, Mezei M, Bieker JJ, Peters LL. Severe anemia in the Nan mutant mouse caused by sequence-selective disruption of erythroid Kruppel-like factor. Proc Natl Acad Sci USA. 2010;107:15151–15156.
115. Arnaud L, Saison C, Helias V, et al. A dominant mutation in the gene encoding the erythroid transcription factor KLF1 causes a congenital dyserythropoietic anemia. Am J Hum Genet. 2010;87:721–727.
116. Kohara H, Utsugisawa T, Sakamoto C, et al. KLF1 mutation E325K induces cell cycle arrest in erythroid cells differentiated from congenital dyserythropoietic anemia patient-specific induced pluripotent stem cells. Exp Hematol. 2019;73:25–37.e8.
117. Varricchio L, Planutis A, Manwani D, et al. Genetic disarray follows mutant KLF1-E325K expression in a congenital dyserythropoietic anemia patient. Haematologica. 2019;104:2372–2380.
118. Mercuriali F, Inghilleri G. Transfusion risks and limitations. Minerva Anestesiol. 1999;65:286–292.
119. Jackson M, Ma R, Taylor AH, et al. Enforced expression of HOXB4 in human embryonic stem cells enhances the production of hematopoietic progenitors but has no effect on the maturation of red blood cells. Stem Cells Transl Med. 2016;5:981–990.
120. Lapillonne H, Kobari L, Mazurier C, et al. Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine. Haematologica. 2010;95:1651–1659.
121. Qiu C, Hanson E, Olivier E, et al. Differentiation of human embryonic stem cells into hematopoietic cells by coculture with human fetal liver cells recapitulates the globin switch that occurs early in development. Exp Hematol. 2005;33:1450–1458.

122. Kobari L, Yates F, Oudrhiri N, et al. Human induced pluripotent stem cells can reach complete terminal maturation: in vivo and in vitro evidence in the erythropoietic differentiation model. Haematologica. 2012;97:1795–1803.

123. Yang CT, French A, Goh PA, et al. Human induced pluripotent stem cell derived erythroblasts can undergo definitive erythropoiesis and co-express gamma and beta globins. Br J Haematol. 2014;166:435–448.