# Exploring the erythroblastic island

Merav Socolovsky

**Two new studies suggest a crucial role for macrophages in boosting the number of red blood cells produced *in vivo* during stress, with translational implications for disease states such as β-thalassemia and polycythemia vera (pages 429–436 and 437–445).**

Mammalian red blood cells (RBCs) are short-lived flexible biconcave discs, optimally adapted for transporting oxygen. They are continuously replenished through a process known as erythropoiesis, where in a span of three to five cell divisions, CFUe (colony-forming unit erythroid) progenitors differentiate into erythroblasts that, in turn, mature and enucleate to form reticulocytes (new RBCs). A negative feedback loop allows stress conditions that threaten tissue oxygen tension, such as anemia or bleeding, to accelerate RBC production (**Fig. 1**). It consists of an oxygen-sensing transcriptional mechanism in the kidney that compares the actual and optimal tissue oxygen levels and generates an 'error' signal in the form of the hormone erythropoiein (Epo), whose magnitude reflects the degree of stress. Epo is an essential viability factor for erythroid progenitors: CFUe and early erythroblasts are continuously generated in large excess, but the fraction of these cells that survives to complete differentiation is determined by Epo levels[1,2].

In culture, adult mammalian CFUe progenitors require no other cell type to fully differentiate into RBCs. However, in hematopoietic tissue, these cells differentiate in the context of a specialized niche, the erythroblastic island, where erythroblasts are attached in concentric rings to one or more central macrophages[3,4]. Progenitor niches have been described for many tissues, providing a locale that concentrates tissue-specific nutritional and autocrine factors and integrates extracellular inputs. Studies *in vitro* suggested similar functions for the erythroblastic island. However, their contribution to erythropoiesis *in vivo* remained unclear. In this issue of *Nature Medicine*, two studies by Chow *et al.*[5] and Ramos *et al.*[6] suggest a key role for the erythroblastic island macrophage in the erythropoietic response to stress conditions such as hemolytic anemia or bleeding. Their findings point to macrophages as potential targets in the treatment of disorders where erythropoiesis is accelerated inappropriately.

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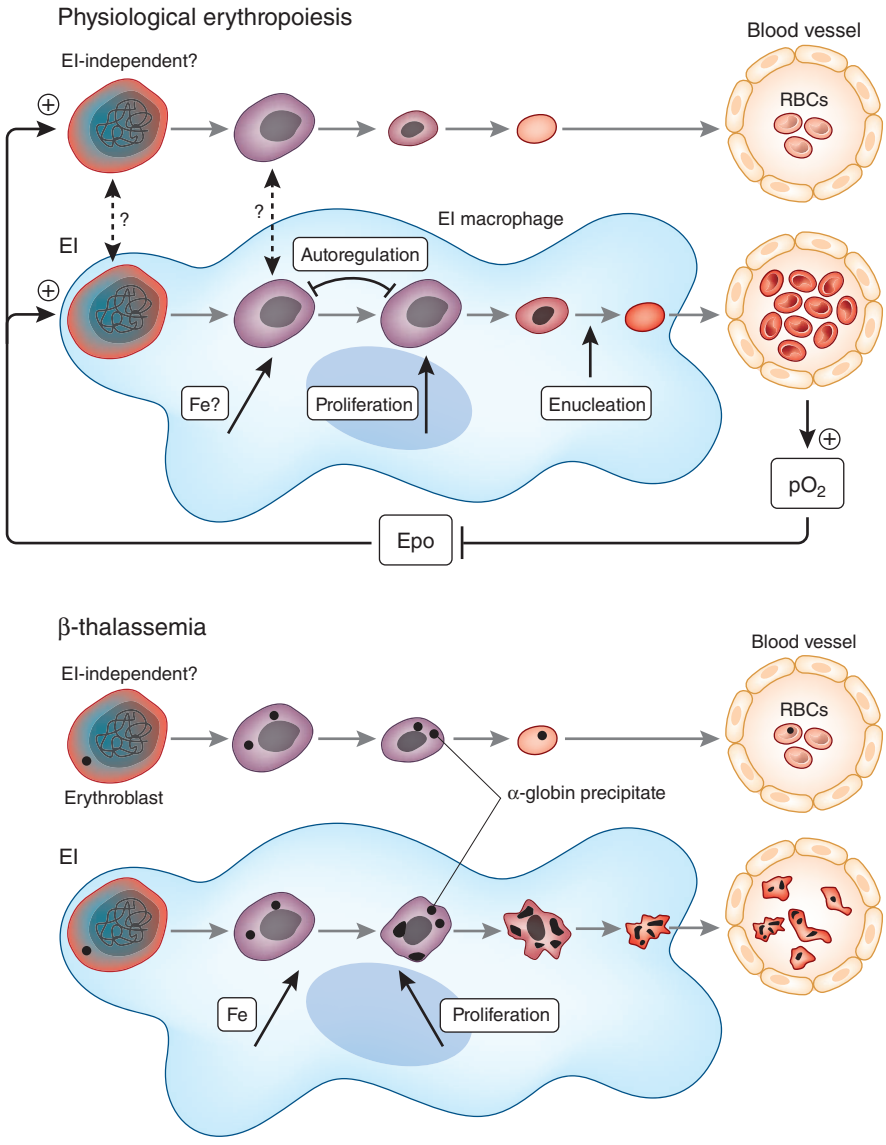


Katie Vicari

**Figure 1** Erythropoiesis within and without the erythroblastic island. Erythroblasts differentiate into RBCs within the erythroblastic island (EI) niche, where they are attached to a macrophage. Differentiation is driven by a negative feedback loop, where tissue oxygen ($pO_2$) regulates the hormone Epo, an essential erythroblast viability factor (top). Macrophages amplify the response to Epo by promoting additional erythroblast cell divisions, assist in enucleation and iron uptake, and hold erythroblasts in close proximity, allowing autoregulatory interactions. It is not known whether erythroblasts repeatedly attach and detach from the erythroblastic island (dashed arrows) or whether they can differentiate entirely outside the erythroblastic island *in vivo*. Chow *et al.*[5] and Ramos *et al.*[6] show that macrophages contribute to the accelerated production of RBCs when $pO_2$ is threatened. In β-thalassemia (bottom), precipitation of excess hemoglobin α-chains (dark inclusions) destroys erythroblasts and RBCs. Paradoxically, macrophage depletion improves this condition, possibly by forcing erythroblastic island–independent erythroblast maturation, with reduced iron uptake and consequently reduced globin chain synthesis, resulting in fewer α-globin precipitates and better RBC quality.

How might the erythroblastic island aid erythropoiesis? In mammalian development, the first wave of primitive (yolk sac) erythroblasts differentiate independently of a niche but are thought to attach to the erythroblastic island during enucleation[7]. Adult erythroblasts similarly enucleate more efficiently in the context of the erythroblastic island[3,4]. However, enucleation is probably not the only erythroblastic island function; indeed, the nonenucleating erythroblasts of birds are closely attached to sinusoidal endoethlial cells[8], suggesting that interaction with niche-supporting cells has a fundamental role in erythroblast differentiation.

The mammalian erythroblastic island is held together by adhesion interactions whose full complement and downstream signaling are not yet clear. The architecture of the erythroblastic island allows for reciprocal autoregulatory interactions between neighboring erythroblasts that stabilize erythropoiesis *in vivo*[2,9]. *In vitro*, erythroblasts that differentiate while attached to macrophages undergo additional cell divisions compared with nonattached erythroblasts, suggesting that macrophages may amplify the response to Epo (**Fig. 1**)[10]. Erythroblastic island macrophages may also directly transfer iron, a rate-limiting nutrient in hemoglobin synthesis, to developing erythroblasts[11].

Are these erythroblastic island functions relevant *in vivo*? Mice lacking integrins, which mediate adhesion interactions within the erythroblastic island, fail to boost erythropoiesis during stress[12]. Similar results were found for mice in which macrophages were chemically depleted[13]. Chow *et al.*[5] and Ramos *et al.*[6] build on the macrophage depletion approach to further examine erythroblastic island function. Both groups also address non–erythroblastic island functions of macrophages that may affect RBC numbers: tissue macrophages take up and destroy old or damaged RBCs; conversely, they promote RBC formation, by recycling the iron derived from ingested RBCs back into the circulation and serving as the major source of iron for developing erythroblasts.

Chow *et al.*[5] conditionally depleted CD169-expressing macrophages in adult mice, and both groups also used a more severe chemical depletion with clodronate-encapsulated liposomes[5,6]. Long-term macrophage depletion produced a mild iron-deficiency anemia, with decreased serum iron and high blood Epo. The response to stress conditions such as bleeding was markedly attenuated: the expected increase in erythroblasts in the bone marrow and spleen was impaired and the increase in blood reticulocytes was sluggish. Notably, both groups went on to show that, in addition to enhancing the appropriate acceleration of erythropoiesis during stress, macrophages sustain disease states where erythropoiesis is increased inappropriately. Thus, macrophage depletion ameliorated the phenotype of a mouse model of polycythemia vera, in which a mutation in the Epo receptor-associated Jak2 tyrosine kinase results in an Epo-independent massive increase in circulating RBCs.

Macrophage depletion produced a dramatic improvement in a mouse model of β-thalassemia[6]—an inherited anemia where reduced expression of the β-globin chain of hemoglobin results in a relative excess of α-globin chains, which precipitate within erythroblasts and RBCs, causing their premature destruction. Paradoxically, the symptoms of this disease are in part caused by attempted but ineffective feedback compensation. High Epo drives a deleterious expansion of erythroid-forming tissue in bones and spleen, which suppresses the iron-regulating hormone hepcidin, increasing iron absorption and ultimately causing toxic iron overload. Macrophage depletion broke this pernicious cycle, substantially reducing splenic erythroblast numbers and spleen size, increasing hepcidin and decreasing iron overload. There was also a marked improvement in anemia: although fewer RBCs were produced, they were of better quality and survived longer in the circulation. Indeed, individual reticulocytes contained less hemoglobin but also less of the destructive α-globin precipitate (**Fig. 1**).

Together, these studies show that macrophages may be an effective new target in the treatment of inappropriately accelerated erythropoiesis. But how do macrophages exert their effects? Are the consequences of macrophage depletion the result of reduced iron availability? Alternatively, are they caused by the loss of interactions within the erythroblastic island niche? These are still open questions. Certainly, reduced iron delivery to developing erythroblasts is a sufficient explanation for failure to accelerate erythropoiesis. It can also explain the remarkable improvement in the phenotype of mice with β-thalassemia. In healthy mice, iron is rate limiting for, and promotes, heme and hemoglobin synthesis. However, in β-thalassemia, iron probably promotes the inherently imbalanced synthesis of the α- and β-globin chains, exacerbating the pathological excess in α-globin and the damage to erythroblasts and RBCs (**Fig. 1**). Indeed, recent work[14,15] shows that restricting erythroblast iron uptake in β-thalassemic mice improves their phenotype in a manner closely similar to that reported here for macrophage depletion.

Based on their data, however, both groups concluded that alterations in systemic iron cannot account for all the consequences of macrophage depletion. Iron supplementation did not block the effects of macrophage depletion, and iron chelation failed to mimic it[5,6]. Intriguingly, although macrophage depletion successfully slowed RBC production during stress even in the presence of high serum iron, the new RBCs that were produced under these conditions contained less hemoglobin[5]. Therefore, in the absence of macrophages, developing erythroblasts seem to experience a shortage of iron, even while its levels in serum are high. This raises the possibility that erythroblastic island macrophages somehow increase delivery of iron to erythroblasts during stress. Macrophages may increase the local niche concentration of iron or may directly transfer iron to erythroblasts[11]. A simple explanation may be that the additional cell divisions undergone by erythroblasts when attached to macrophages[10] provide more time for iron uptake and hemoglobin synthesis (**Fig. 1**).

The new studies show the importance of macrophages for RBC production *in vivo* but also highlight large gaps in our knowledge. How do we distinguish between erythroblastic island–dependent and erythroblastic island–independent macrophage functions? At present, no single cell surface marker uniquely distinguishes erythroblastic island macrophages from other resident tissue macrophages. Intriguingly, severe macrophage depletion caused only a mild deficit in basal RBC production[5,6]. Is this the result of a few remaining erythroblastic islands working 'overtime', or does it instead reflect erythroblastic island–independent erythropoiesis *in vivo* (**Fig. 1**)? In support of the latter possibility, erythroblasts of all maturational stages were displaced into the bloodstream by macrophage depletion[5], which is reminiscent of yolk-sac erythroblasts and newborn erythroblasts, which are routinely found unattached to the erythroblastic island. Might erythroblastic island–independent differentiation be a normal component of adult erythropoiesis *in vivo*, and if so, what might be its function?

Observations of unattached erythroblasts *in vivo* support the idea of a dynamic exchange in which erythroblasts repeatedly attach and detach from their niche[4] (**Fig. 1**). What might determine the size of the niche space or its occupancy by erythroblasts? How does the niche attract erythroblasts, and what might pull them away? Can niche space be manipulated by means other than macrophage depletion? The studies by Chow *et al.*[5] and Ramos *et al.*[6] suggest that the erythroblastic island is directly implicated in both physiological and pathological erythropoiesis and may serve as an effective therapeutic target. Ultimately, a comprehensive

understanding of erythroblastic island functions will require a quantitative systems approach that integrates its multiple components including its role in iron homeostasis, adhesion interactions, effects on cycling and differentiation and multiple feedback interaction within and outside the niche.

**COMPETING FINANCIAL INTERESTS**
The author declares no competing financial interests.

1. Koury, M.J. & Bondurant, M.C. *Science* **248**, 378–381 (1990).
2. Koulnis, M., Liu, Y., Hallstrom, K. & Socolovsky, M. *PLoS ONE* **6**, e21192 (2011).
3. Manwani, D. & Bieker, J.J. *Curr. Top. Dev. Biol.* **82**, 23–53 (2008).
4. Mohandas, N. & Chasis, J.A. *Transfus. Clin. Biol.* **17**, 110–111 (2010).
5. Chow, A. *et al. Nat. Med.* **19**, 429–436 (2013).
6. Ramos, P. *et al. Nat. Med.* **19**, 437–445 (2013).
7. McGrath, K.E., *et al. Blood* **111**, 2409–2417 (2008).
8. Sorrell, J.M. & Weiss, L. *Am. J. Anat.* **164**, 57–66 (1982).
9. Socolovsky, M. *et al. PLoS Biol.* **5**, e252 (2007).
10. Rhodes, M.M., Kopsombut, P., Bondurant, M.C., Price, J.O. & Koury, M.J. *Blood* **111**, 1700–1708 (2008).
11. Leimberg, M.J., Prus, E., Konijn, A.M. & Fibach, E. *J. Cell. Biochem.* **103**, 1211–1218 (2008).
12. Ulyanova, T., Jiang, Y., Padilla, S., Nakamoto, B. & Papayannopoulou, T. *Blood* **117**, 975–985 (2011).
13. Sadahira, Y. *et al. J. Leukoc. Biol.* **68**, 464–470 (2000).
14. Li, H. *et al. Nat. Med.* **16**, 177–182 (2010).
15. Gardenghi, S. *et al. J. Clin. Invest.* **120**, 4466–4477 (2010).

# Love the one you're with: the HIV, B cell and $T_{FH}$ cell triangle

Shiv Pillai

**Many HIV-infected people show impaired humoral immune responses, but it is unclear why. A new view into this conundrum may be provided with the recent discovery of altered interactions between follicular helper T ($T_{FH}$) cells and germinal center B cells from HIV-infected individuals. This leads to inadequate $T_{FH}$ cell help for germinal center B cells and decreased B cell antibody responses (pages 494–499).**



The immune system is intensely xenophobic and cruel. It allows us to survive in a hostile environment by devouring pathogens, torching them with reactive chemicals, destroying the cells they infect and sometimes making holes in their membranes to blow them into smithereens. The continuous game played between host and virus is, however, not one in which the latter seek to eliminate their seemingly pitiless hosts. Viruses are not vengeful, and it is not in their interest to be deprived of the substrate that sustains them, especially because transmission to another host can be risky.

Viruses rapidly evolve to evade our protective responses by mutating antibody and cytotoxic T cell epitopes encoded in their genomes. We, too, initiate a mutational mechanism whereby antigen-activated B lymphocytes in germinal centers go through a Darwinian process of genetic diversification followed by natural selection and survival of the fittest. As a result, we can generate antibodies of extraordinarily high affinity that not only protect us from subsequent infections but also attenuate the spread of virus during an ongoing infection.

In a T cell–dependent immune response to a virus, dendritic cells may present a peptide from a specific pathogen-derived protein antigen and activate naive $CD4^+$ helper T cells in the T cell zone. These activated T cells proliferate, are induced to express CD40L and migrate toward the follicle, where B cells reside. A specific B cell internalizes the same protein antigen that was originally presented by dendritic cells and meets activated T cells at the T cell–B cell interface. Activation of the B cell via CD40L on the helper T cell results in its proliferation and some differentiation. The activated B cells may, in turn, induce previously activated T cells to differentiate and in a Bcl-6 transcription factor–dependent manner express high amounts of the CXCR5 chemokine receptor, the inducible co-stimulatory receptor and the programmed death-1 (PD-1) inhibitory receptor. These polarized $CD4^+$ T cells are called $T_{FH}$ cells. They secrete interleukin-21 (IL-21) and other cytokines, migrate following a CXCL13 chemokine gradient into the follicle, invite in a few activated B cells and orchestrate the germinal center reaction. In this reaction, $T_{FH}$ cells induce germinal center B cells to proliferate and form the dark zone of the germinal center, where they undergo extensive somatic hypermutation. After this, high-affinity B cells are selected by $T_{FH}$ cells in the light zone of the germinal center, where they differentiate into memory B cells or into the precursors of long-lived plasma cells.

Individuals with HIV/AIDS often have dysfunctional humoral immune responses, and their memory B cells often show features of exhaustion[1,2]. Why exactly such defects in humoral immune responses develop has until recently not been clear. Studies from the Streeck and Koup laboratories demonstrated that there are expanded numbers of $T_{FH}$ cells in patients with AIDS and in monkeys infected with SIV[3,4]. It has been suggested[5] that an increase in $T_{FH}$ cell numbers perhaps contributes to the aberrant selection of unwanted lower-affinity B cells and thereby leads to humoral immune dysfunction, but currently there is no direct evidence to support this notion. The high number of $T_{FH}$ cells seen in patients with AIDS may well reflect robust T and B cell activation because of viral persistence. In a recent study, Perreau *et al.*[6] also suggested that $T_{FH}$ cells serve as a major site of HIV replication. The finding that $T_{FH}$ cells are important reservoirs of the virus may not be too surprising when one takes into account that these cells represent the most abundant effector $CD4^+$ T cell population in HIV-infected individuals.

In this issue of *Nature Medicine*, Cubas *et al.*[7] provide a new mechanistic explanation for the humoral immune dysfunction seen in many people with AIDS. They show that, in comparison to healthy control people, a greater proportion of germinal center B cells in the lymph nodes of HIV-infected subjects express high amounts of PD-L1, one of the ligands for the PD-1 inhibitory receptor. They also show that PD-1 engagement on $T_{FH}$ cells leads to an impairment of IL-21 secretion and attenuation of B cell antibody responses. B cell functionality can be restored by the exogenous addition of IL-21 or by blocking PD-1 signaling **(Fig. 1)**. How exactly HIV infection of T cells enhances PD-L1 on germinal center B cells is unclear, but as the authors suggest, this is likely to be a consequence of antigen overload and a more active immune response. Indeed the promoter

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