Is the erythropoietin receptor the key to the identification of the central macrophage in erythroblastic islands?

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Erythroblastic islands (EBI) are niches for mammalian erythropoiesis.[1] They were first identified in 1958 by Marcel Bessis and consist of a group of differentiating erythroblasts surrounding a central macrophage.[2] Over the years, a lot of attention has been paid to understand the role of the central macrophage in regulating the differentiation of the maturing erythroblasts within these islands and in defining the surface proteins that mediate the interaction between the erythroblasts and the central macrophage.[3–14] While some progress has been made in these areas of investigation, the definitive identity of this central macrophage and its function is yet to be fully characterized.

In a recent study published in *Blood*, Li et al shed some further light on the identity of the central macrophage of EBI and surprisingly highlighted the expression of the erythropoietin receptor (Epo-R) on these cells.[15] The authors began the study by reasoning that (i) Epo-R expression on erythroid cells is essential for terminal erythroid maturation and (ii) Epo-R expression has been identified in various cell types besides the erythroid lineage, and as such it may also be expressed by the central macrophage to ensure efficient erythropoiesis. To test this intriguing hypothesis, the authors used a knock-in mouse model, in which the Epo-R is fused to the green fluorescent protein (GFP) reporter (Fig. 1). In the original description of this mouse model, it was reported that while more than 90% of the cells that were GFP$^+$ were found in the Ter119$^+$ population, ~2.5% of the GFP$^+$ cells were found in the Ter119$^-$ cell population.[16] It was suggested that these GFP$^+$ cells and Ter119$^-$ could represent earlier erythroid progenitors that have yet to express Ter119, since GFP expression was seen only in erythroid lineage and not in any other hematopoietic cell lineages. Interestingly, in the present study, using the same murine model, it was noted that about 5% of the macrophage population in the bone marrow express the Epo-R transgene and that this specific macrophage population efficiently form EBIs contrary to the ones that do not express the transgene. Indeed, following isolation of bone marrow EBIs, it was documented by imaging flow cytometry that the central macrophage within the island was GFP positive, while non-GFP populations of macrophages could not efficiently form EBIs. Thus, using Epo-R expression as a means of discrimination between subpopulations of macrophages, the authors could establish a novel and refined characterization of the macrophage supporting erythropoiesis. Interestingly, even within this population of Epo-R$^+$ macrophages, they found significant heterogeneity with regard to the expression of different surface markers expressed by the macrophages.[15] While most or all of the F4/80$^+$Epo-R$^+$ are CD45$^+$, VCAM1$^+$ CD169$^+$, and ER-HR3$^+$, only 69% are Ly6C$^+$ and 35% are CD163$^+$. These findings suggest that there is considerable heterogeneity in the F4/80$^+$Epo-R$^+$ macrophages and additional surface markers may be needed for further characterization of the EBI macrophages.

Of note, the authors did not limit their studies exclusively to the murine system. They isolated erythroblastic islands from human fetal liver and were able to recreate EBIs in vitro. Using an antibody against the Epo-R for the human studies, they documented the presence of the Epo receptor, mostly on the surface of erythroblasts along with some cytoplasmic staining of central macrophage.[15]

Several fascinating unanswered questions arise from this very interesting study. It is first worth noting that it is surprising that the differentiating murine erythroblast surrounding the central macrophage appear to be negative for eGFP, implying that they express little or no Epo-R compared to the macrophage. This finding, if true, would shift the paradigm that in the mouse, the Epo-R is only expressed at the erythroid progenitor stages and is dramatically downregulated during terminal erythroid differentiation. It also highlights a major difference between human and murine erythropoiesis, since, as shown in the present study, late stage terminally differentiating human erythroblasts are positive for Epo-R when stained with a specific antibody. Another interesting caveat that caught our attention is that while top regulated pathways in Epor-eGFP$^+$ macrophages included genes involved in erythrocyte development and in receptor-mediated endocytosis, it did not include the Epo signaling pathway.

Another interesting issue that needs further attention is the possibility that the Epo-R GFP observed in the central macrophage is the result of remnants of phagocytosis of erythroblasts and/or pyrenocytes since the primary function of central macrophage is phagocytosis of apoptotic cells and

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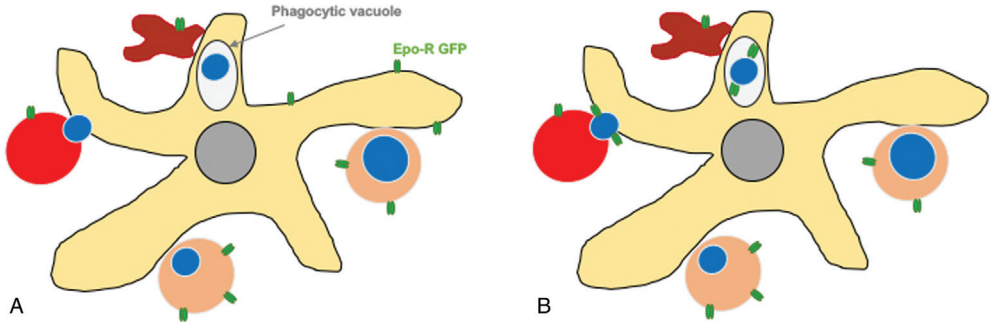

Figure 1. Two potential explanations for the presence of the Epo-R in the central macrophage. (A) As hypothesized by Li et al, it could be that in erythroblastic islands, the Epo-R GFP is located on the surface of both the differentiating erythroblasts and the central macrophage. (B) A potential alternative explanation is that the Epo-R GFP could result from phagocytosis of erythroblasts and/or pyrenocytes and accumulates within phagocytic vacuoles in the macrophage.

pyrenocytes.[17,18] Some support for this possibility comes from the extensive phagocytosis documented in the Epor-eGFP$^+$ macrophages of fetal liver in the reported study.

While the issues raised by us regarding the present study need to be critically addressed in future studies to validate the role of Epo-R in the central macrophage in regulating erythropoiesis, they do not distract from the novelty of the reported findings. Indeed, the F4/80$^+$Epo-R GFP$^+$ macrophages can still be used as a resource to help further define and functionally characterize these fascinating cells that nurse differentiating erythroblasts that have remained a mystery for so long.

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