

Erythroblast island macrophages: recent discovery and future perspectives

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Abstract

Erythroblastic island (EBI), composed of a central macrophage surrounded by developing erythroid cells, is a structure found in hematopoietic tissues such as fetal liver and bone marrow. It is the first described hematopoietic niche that predominantly supports erythropoiesis. Although it is well accepted that EBIs and EBI macrophage play important roles during erythropoiesis, the mechanisms by which they support erythropoiesis remain largely unclear due to our inability to identify and isolate EBI macrophages. Earlier efforts to identify surface markers for EBI macrophages have focused on the adhesion molecules which are involved in macrophage's interaction with erythroblasts. These include EMP, Vcam1, CD169, CD163, and α V integrin. Findings from these earlier studies suggested that combination of Vcam1, CD169, and mouse macrophage surface marker F4/80 can be used to define mouse EBI macrophage. We found that not all F4/80⁺Vcam1⁺CD169⁺ macrophages are EBI macrophages. Instead, we discovered that EBI macrophages are characterized by the expression of Epor in both mouse and man. RNA-seq analyses of the newly identified EBI macrophages revealed that EBI macrophages have involved specialized function in supporting erythropoiesis. Our findings provide foundation for future studies. Here we will review current knowledge of EBI macrophages and discuss future perspectives.

Keywords: Epor, Erythroblast islands, Erythroblastic island macrophages, Erythropoiesis

1. INTRODUCTION

Erythropoiesis is a complex process by which mature red cells are generated from hematopoietic stem cells and can be subdivided into three stages: early stage erythropoiesis, terminal erythroid differentiation, and reticulocyte maturation. Defects at any stage during this process can lead to disordered erythropoiesis.^{1–3} These include Cooley anemia,^{4,5} congenital dyserythropoietic anemia,^{6–8} Diamond–Blackfan anemia,^{9,10} malarial anemia,^{11–13} myelodysplastic syndromes,^{14–16} and polycythemia *vera*.^{17–20} Thus, a better and detailed understanding of the process of erythropoiesis is of both biological and clinical significance. Previous studies on the regulations of erythropoiesis have been primarily focused on cell intrinsic factor of erythroid cells, such as erythropoiesis master regulator transcription factors GATA binding protein 1 (GATA1) and Krueppel-like factor 1

(KLF1) as well as erythropoietin (EPO) and its receptor (EPOR) mediated signal transduction.^{21–25}

In addition to cell intrinsic regulations, erythropoiesis is also influenced by niche and microenvironment. It has been well established that erythropoiesis occurs at the erythroblastic island (EBI) which is composed of a central macrophage surrounded by developing erythroblasts. EBI was first defined in 1958 by Marcel Bessis, based on the analysis of transmission electron micrographs of sections of bone marrow.²⁶ Based on the structural observations, Bessis et al made a number of interesting inferences concerning the role of central macrophages. It was suggested that the macrophage functions as a “nurse” cell, providing iron to developing erythroblasts for heme synthesis.²⁷ The study of EBI was extended in 1970s by reconstruction of three-dimensional scale models of bone marrow.²⁸ Later studies further confirmed the existence of EBIs in the mammal bone marrow (BM), spleen and fetal liver (FL), but not in yolk sac.^{29,30} It was proposed that the macrophages in the center of EBIs not only transport iron to erythroblasts for the synthesis of hemoglobin, they also secrete many cytokines to support the survival and differentiation of erythroblasts,^{29,31,32} as well as act as phagocytes to engulf and degrade erythroblast nuclei during nuclear extrusion.^{33–35} Despite extensive studies on EBIs over the past few decades, the identity of EBI macrophage has remained elusive until very recently.³²

The lack of inability to identify and isolate EBI macrophages prospectively has hindered the investigation on EBI macrophages. Very recently, our group discovered that EBI macrophages are characterized by the expression of Epor in both mouse and human. We further performed RNA-seq on the newly identified EBI macrophages. Bioinformatic analyses revealed that

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EBI macrophages have involved specialized function in supporting erythropoiesis and suggested potential underlying molecular mechanisms. These findings provide solid foundation for many future studies.³² In this review, we will provide an overview of identification and characterization of EBI macrophage. We will also discuss future perspectives.

2. IDENTIFICATION OF EBI MACROPHAGE

2.1. Adhesion molecules involved in EBI formation

EBI formation involves the interaction between EBI macrophages and erythroblasts. Therefore, earlier efforts to identify surface markers for EBI macrophages were focused on the adhesion molecules. Erythroblast macrophage protein (EMP) was the first molecule identified on both erythroblast and EBI macrophage that mediates the association between macrophages and erythroblast attachments through hemophilic interaction.³⁶ In EBI cultures, absence of EMP leads to aberrant erythropoiesis and increased levels of apoptosis, suggesting that the direct association between the EBI macrophage and the erythroblasts is essential for erythroid maturation and prevention of cell death.³⁷ However, a recent study demonstrated that EMP expressed by macrophages but not by erythroblasts is important for the EBI formation.³⁸

The other receptor/counter receptor identified as mediating cell-cell interactions within EBI were VCAM1 in EBI macrophages and $\alpha4\beta1$ integrin in erythroblasts.³⁹ The biological significance of this interaction is underlined in the experiment in which the formation of EBI was disrupted by the application of antibodies against either $\alpha4\beta1$ integrin or VCAM-1.³⁹ This receptor/counter receptor interaction also contributes to the integrity of EBIs during primitive erythropoiesis.^{40,41} However, the lack of phenotypic changes of *Vcam1*^{-/-} mice questions the physiological role of *Vcam1* in erythropoiesis.³⁸

In addition, the adhesion molecule αV integrin expressed on macrophage and ICAM4 expressed on erythroblasts also plays critical roles in maintaining EBI integrity, disrupting the binding of αV integrin, and ICAM4 leads to decreasing number of EBI.^{42,43}

The other adhesion molecule expressed by EBI macrophage is CD169. It has been shown that CD169 localizes at the site of macrophage/erythroblast contact in EBIs.⁴⁴⁻⁴⁷ A recent study shows that depletion of CD169⁺ macrophages in mice leads to impaired erythropoiesis *in vivo*.⁴⁸ It should be noted that the counter receptor for CD169 on erythroblasts has yet to be identified.

A number of additional macrophage surface proteins have been identified as receptors for erythroblasts. One macrophage adhesion glycoprotein with an unidentified erythroid binding partner is CD163 (formerly called ED2 antigen), which functions

as a receptor for hemoglobin-haptoglobin complexes and is involved in the clearance of free hemoglobin.⁴⁹ CD163 contains an erythroblast adhesion motif as well, which mediates binding of macrophages to erythroid precursors facilitating erythroblast expansion and survival.⁵⁰ Other molecules that have been reported to be expressed on EBI macrophages are lectin-like sheep erythrocyte receptor, erythroblast receptor (EbR), ED2, CD206, ER-HR3, Ly6G, tyrosine kinase receptor MerTK, and Axl.⁵¹⁻⁵³

2.2. Use of imaging flow cytometry to characterize EBI macrophages

In 2017, Seu et al used a novel method, multispectral imaging flow cytometry (IFC), to characterize EBI macrophages. IFC combines the high-throughput advantage of flow cytometry with the morphological and fluorescence features derived from microscopy.⁵⁴ This method provides quantitative analysis of EBIs, as well as structural and morphological details of the EBI macrophages and associated cells. Using IFC, Seu et al showed that consistent with previous findings, F4/80, VCAM1, and CD169 are expressed on EBI macrophages. However, CD163 is not expressed on mouse EBI macrophages even though it is expressed on rat EBI macrophages. Intriguingly, CD11b is not expressed on EBI macrophages in mice, although it is abundantly expressed on myeloid cells within the islands.

2.3. EBI macrophages are characterized by the expression of Epor

Based on previous findings, it is thought that combination of F4/80, *Vcam1*, and CD169 can be used to define EBI macrophage. However, by examining the ratio of F4/80⁺*Vcam1*⁺CD169⁺ macrophages and erythroblasts, we found that the ratio of F4/80⁺*Vcam1*⁺CD169⁺ versus erythroblasts in mouse bone marrow is only about 1:2.6. It has been reported that the number of erythroblasts per island ranges from 10 cells observed in tissue sections from rat femur to 5 to >30 erythroblasts in islands harvested from human BM. Thus, our findings strongly suggest that it is unlikely all F4/80⁺*VCAM1*⁺CD169⁺ macrophages are EBI macrophages.

Given the facts Epo/Epor is essential for erythropoiesis and that Epor is expressed in numerous non-erythroid cells including macrophages,⁵⁵⁻⁵⁸ we hypothesized that Epor is expressed on EBI macrophages so that Epo can act on both erythroid cells and EBI macrophages to ensure efficient red cell production. To test this, we first used Epor-eGFP knockin mouse model.³² We show that a subpopulation of macrophages in mouse bone marrow and fetal liver express Epor. Importantly, the EBIs are predominantly formed by the Epor⁺ macrophages in both mouse and man. The representative images of EBI are shown as Figure 1. Furthermore, using flow cytometry and IFC, we characterized the surface

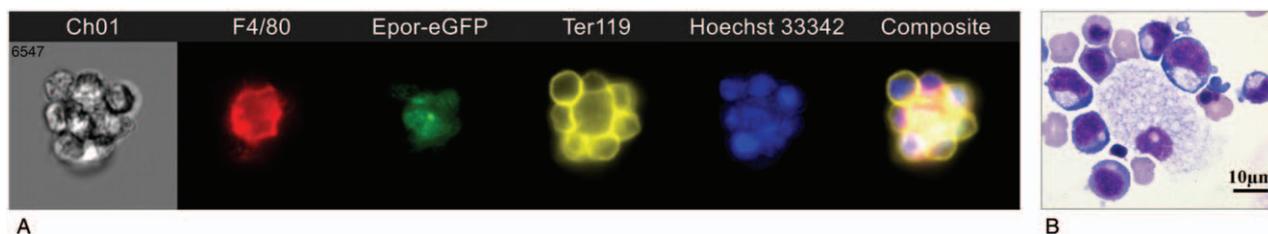


Figure 1. Images of EBI. (A) Native mouse bone marrow EBI revealed by imaging flow cytometry. F4/80: mouse macrophage marker; Ter119, mouse erythroid cell marker. (B) Cytospin image of *in vitro* formed human EBI.

Table 1**Expression of surface markers on EBI macrophages in human, mouse and rat.**

Surface marker	Human	Mice	Rat
F4/80	—	+	—
EPOR	+	+	+
CD45	+	+	+
VCAM1	+	>90%	Unknown
CD169	+	>90%	Unknown
AXL	+	>90%	Unknown
MERTK	+	>90%	Unknown
TIM4	+	>90%	Unknown
ER-HR3	Unknown	~90%	Unknown
Ly6C	—	~69%	—
CD163	+	~35%	+
CD206	+	~15%	Unknown
CD11b	+	Moderate	+
Ly6G	—	—	—

CD=cluster of differentiation.

Note: Expression patterns of AXL, MERTK, and CD206 are our unpublished data.

expression of previously described EBI macrophages. These results along with previous findings are summarized in Table 1. Notably, although CD163 is a marker for human and rat EBI macrophages, it is only expressed on about 35% of mouse EBI macrophages, indicating differences among species.³²

3. FUTURE PERSPECTIVE

Since the discovery of the EBIs more than 60 years, the major gap in the understanding of EBI has been what is the identity of EBI macrophage? Our very recent identification of the EBI macrophages and transcriptomic analyses of the EBI macrophages not only fill this important gap but also offer the opportunity to begin to fill the gaps in mechanistic understanding of the roles of EBI macrophages in normal as well as disordered erythropoiesis. Specifically, with the finding that EBI macrophages are characterized by the expression of Epor, the question is what is the role of Epo/Epor in EBI macrophages? Another question is that although it has long been postulated that EBI macrophages could provide nutrients locally to the surrounding erythroid cells, so far there was no study to demonstrate this is the case. With the iron being the most needed nutrient for erythropoiesis, do EBI macrophages provide iron locally to the developing erythroid cells? Our finding that molecules involved in iron recycle such as PS receptor Tim4, tyrosine kinase MerTK, Axl, heme oxygenase HO-1, iron exporter ferroportin, and iron transporter transferrin are abundantly expressed in EBI macrophages strongly suggest that EBI macrophages could provide iron locally to support erythropoiesis.³² Similarly, it has been thought that EBI macrophages may secrete cytokines to promote erythropoiesis. However, it remains unknown which erythropoiesis-promoting cytokines they secrete. Our finding that erythropoiesis-promoting cytokine Igf1 is expressed in EBI macrophages but not non-EBI macrophages raises the question of whether local secretion of Igf1 by EBI macrophages contributes to erythropoiesis.

Other knowledge gaps include but are not limited to: first, erythroblast enucleation is the last step of terminal erythroid maturation. Do EBI macrophages promote enucleation? If so, does Epo play a role?; second, one function of EBI macrophage is to engulf extruded nuclei. Does Epo enhance engulfment of nuclei by EBI macrophages? If so, what are the underlying molecular

pathways?; third, in addition to iron, do EBI macrophages provide other nutrients to developing erythroid cells? If so, what are these nutrients and what are the routes these nutrients are transferred from EBI macrophages to erythroid cells?; fourth, fetal liver erythropoiesis is different from adult BM erythropoiesis in that FL erythropoiesis is a kind of stress erythropoiesis. Do EBI macrophages contribute to the differences between FL and BM erythropoiesis. If so, to what extent and how? Our ability to identify and isolate EBI macrophages as well as the RNA-seq database we have generated provide the foundation for many future studies to fill these gaps.

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