Erythroblastic Islands: Specialized Microenvironmental Niches for Erythropoiesis

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ABSTRACT

Purpose of review

Erythroblasts differentiate within specialized niches, termed erythroblastic islands, composed of erythroblasts surrounding a central macrophage. This review focuses on our current understanding of molecular mechanisms operating within islands including cell-cell adhesion, regulatory feedback and central macrophage function.

Recent findings

Erythroblasts express a variety of adhesion molecules and recently two interactions have been identified which appear to be critical for island integrity. Erythroblast macrophage protein (Emp), expressed on erythroblasts and macrophages, mediates cell-cell attachments via homophilic binding. Erythroblast intercellular adhesion molecule-4 (ICAM-4) links erythroblasts to macrophages through interaction with macrophage α_V integrin. In ICAM-4 knockout mice erythroblastic islands are markedly reduced, while the Emp null phenotype is severely anemic and embryonic lethal. Perturbation in macrophage differentiation also severely affects erythropoiesis. Retinoblastoma tumor suppressor (Rb) protein stimulates macrophage differentiation by counteracting inhibition of Id2 on PU.1, a transcription factor that is a crucial regulator of macrophage differentiation. Rb deficient macrophages do not bind Rb null erythroblasts and the Rb null phenotype is anemic and embryonic lethal. Lastly, an important signal for phagocytosis of expelled nuclei has been identified. Extruded nuclei rapidly expose phosphatidylserine (PS) on their surface, providing a recognition signal similar to apoptotic cells.

ABSTRACT (CONTINUED)

Summary

Although our understanding of molecular mechanisms operating within islands is at an early stage, tantalizing evidence suggests that erythroblastic islands are specialized niches where intercellular interactions in concert with cytokines play critical roles in regulating erythropoiesis.

Keywords

erythroblastic islands, erythropoiesis, macrophage, erythroblast macrophage protein (Emp), intercellular adhesion molecule-4 (ICAM-4), Rb protein, GATA-1

INTRODUCTION

Mammalian erythroblasts proliferate, differentiate and enucleate within specialized microenvironmental niches, termed erythroblastic islands [1, 2, 3, 4]. Since we are approaching the 50th anniversary of the first description of erythroblastic islands by Dr. Marcel Bessis, it seems timely to focus a review on our current knowledge of the molecular characterization and functions of these compartments dedicated to terminal erythroid differentiation. Fetal liver, bone marrow and splenic erythropoiesis occur within erythroblastic islands, composed of developing erythroblasts surrounding a central macrophage (Figure 1) [2]. Areas of erythropoiesis in long term bone marrow cultures also contain central macrophages [1]. Reconstruction of 3-dimensional models of bone marrow from serial sections show that islands are distributed over the entire marrow space and not localized to specific regions [4]. Although early electron microscopic analysis of thin sections suggest that central macrophages phagocytose extruded nuclei and supply iron to developing erythroblasts [5], the role of central macrophages in iron metabolism is yet to be clearly delineated. Extensive cell-cell interactions, both erythroblast-macrophage, as well as erythroblast-erythroblast occur within islands and, to date, several specific intercellular adhesive interactions have been defined. Although our current understanding of molecular mechanisms operating within islands is at an early stage, tantalizing evidence suggests that erythroblastic islands are highly specialized hematopoietic tissue subcompartments where intercellular interactions in concert with stimulatory and inhibitory cytokines play critical roles in regulating erythropoiesis and apoptosis.

CELLULAR COMPOSITION AND STRUCTURE OF ISLANDS

During steady state erythropoiesis, erythroblastic islands are composed of erythroid cells at various stages of differentiation from CFU-E through young, multilobulated reticulocytes [6, 7, 8, 9, 10]. In the unstressed state there also appears to be variation in the number of cells per island. Tissue sections from rat femur reveal about 10 cells per island [10] while islands harvested from human marrow contain 5->30 erythroblasts per island [8].

In hematopoietic tissues, central macrophages of erythroblastic islands are resident macrophages that have differentiated from monocyte precursors. Hence bone marrow contains monocytes/macrophages at various stages of differentiation and phenotype. Although phenotypic analysis of central macrophages is far from complete in any species on a genomic or proteomic level, certain specifics have been delineated. Currently, the two most useful markers for distinguishing mouse central macrophages from other stromal cells in hematopoietic tissues are F4/80 antigen and Forssman glycosphingolipid. F4/80 antigen is a cell surface glycoprotein with homology to the G-protein linked Transmembrane 7 hormone receptor family [11, 12, 13]. Forssman glycosphingolipid distinguishes central macrophages from monocytes and from bone marrow macrophages cultured with macrophage colony-stimulating factor (M-CSF) [14, 15, 16]. Immunophenotyping of central macrophages harvested from human bone marrow islands shows expression of FcRI, FcRII, FcRIII, CD4, CD31, CD11a, CD11c, CD18, CD31 and HLA-DR [8].

Erythroblastic islands are present in mammalian bone marrow and in splenic red pulp in certain species, such as mouse. In mouse fetal liver, definitive erythropoiesis also occurs in islands [17] but the question of whether primitive erythropoiesis does remains unanswered. In contrast to definitive erythropoiesis, primitive erythroblasts are released into circulation as nucleated red cells and undergo enucleation while in circulation [18]. If the central macrophage has a functional role in enucleation of definitive fetal-, bone marrow- and splenic-derived erythroblasts, the observation that primitive erythroblasts do not enucleate during differentiation in the liver argues that they may not differentiate within islands. In support of this speculation is a report showing that mature avian red cells, that remain nucleated, differentiate in bone marrow that does not contain islands [19].

CELL-CELL ADHESION

Although erythroblasts express a variety of cell adhesion molecules only a few involved in cell attachments within erythroblastic islands have been delineated to date. A 36kDa transmembrane protein Emp (erythroblast macrophage protein) has been discovered on both

erythroblast and macrophage surfaces, that appears capable of mediating erythroblast-macrophage or erythroblast-erythroblast attachments via homophilic binding [3]. The cytoplasmic domain of Emp contains several potential binding sites for SH2 domains and a potential binding site for phosphotyrosine binding domain, suggesting signaling function. When erythroblasts are cultured in the absence of macrophages or in the presence of anti-Emp a six-fold increase in apoptosis is observed, accompanied by a marked decrease in erythroid cell proliferation, maturation, and enucleation [20]. A recent report has shown that Emp null fetuses are severely anemic and die in utero [21*], strongly supporting a crucial role for Emp in erythropoiesis. A second identified adhesive interaction within islands involves erythroblast/central macrophage binding mediated by erythroblast $\alpha_4\beta_1$ integrin and its counterreceptor on central macrophages, VCAM-1 [22]. Antibodies to either $\alpha_4\beta_1$ or VCAM-1 disrupt island integrity.

The final adhesive interaction currently characterized entails erythroid intercellular adhesion molecule-4 (ICAM-4) binding to macrophage α_V integrin. Blocking ICAM-4/α_V binding with α_V synthetic peptides produces a 70% decrease in islands reconstituted in vitro [23]. Moreover, a striking decrease in islands reconstituted from marrow of ICAM-4 null mice or formed in vivo in these knockout mice is observed [24*]. Collectively, these data provide convincing evidence that ICAM-4/α_V adhesion participates in island formation. Interestingly, a secreted isoform of mouse ICAM-4, ICAM-4S, that is upregulated late in terminal differentiation, has been discovered, suggesting a role during this stage of erythropoiesis [25]. ICAM-4S may compete with membrane bound ICAM-4 for integrin counterreceptors, thereby blocking ICAM-4/α_V adhesion. This potential inhibitory function of ICAM-4S could facilitate detachment of young reticulocytes from erythroblastic islands, thereby enabling their movement into the vasculature.

Several other macrophage receptors for erythroblasts have been described, however the erythroblast counterreceptors have not yet been identified. Macrophages express a membrane glycoprotein diffusely localizing to areas of contact with erythroblasts within erythroblastic

islands [26]. This macrophage protein, originally called SER (receptor for sheep erythrocytes) but now termed sialoadhesion, binds sialylated glycoconjugates [27, 28]. Finally, binding of rat erythroblasts to bone marrow macrophages is inhibited by antibodies to ED2 antigen, a macrophage surface component yet to be characterized [29].

Future investigations are likely to uncover additional adhesive interactions between cellular components of erythroblastic islands. We can postulate that the various linkages within islands are dynamic during erythroid development. A key unanswered question is what is the role of these adhesive molecular attachments? Given that integrin linkages with the actin cytoskeleton regulate intracellular signaling (as reviewed by [30]) certain of these receptor-counterreceptor interactions may trigger signaling pathways coordinating adhesion and gene expression.

REGULATORY FEEDBACK WITHIN ISLANDS

It is appealing to speculate that erythropoiesis is driven by both positive and negative regulatory feedback mechanisms operating within islands. In support of this thesis, Fas/Fas ligand regulation of apoptosis apparently occurs [31]. Erythroblasts express Fas throughout terminal differentiation, however, Fas crosslinking transduces a death signal in immature but not in mature erythroblasts. Hence mature erythroblasts expressing Fas are not susceptible to Fas ligand binding. Late in differentiation Fas ligand is induced and orthochromatic erythroblasts demonstrate a Fas-based cytotoxicity against Fas expressing immature erythroblasts. Importantly, elevated erythropoietin levels, consistent with those present in anemic individuals, protect early erythroblasts from Fas-induced cytotoxicity by late erythroblasts. This mechanism would upregulate erythropoiesis in response to increased erythropoietin by enabling a greater number of immature erythroblasts to complete terminal differentiation. Another feedback potentially operating between cellular components of islands involves RCAS1 (receptor binding cancer antigen expressed in SiSo cells) and its receptor [32]. Immature erythroblasts express RASC1 receptor. Soluble RCAS1, secreted by bone marrow macrophages, activates pro-apoptotic caspases 8 and 3 in immature erythroblasts. Collectively, these findings delineate

possible mechanisms for negative regulatory feedback between mature and immature erythroblasts and between macrophages and immature erythroblasts.

Erythroblasts differentiating in vivo or in vitro secrete two angiogenic factors, vascular endothelial growth factor A (VEGF-A) and placenta growth factor (PIGF) [33]. Media from cultured erythroblasts induces migration of monocytes and endothelial cell permeability, both inhibited by VEGF-A and PIGF specific antibodies. Since erythroid progenitors do not express receptors for either of these angiogenic factors, the secreted molecules may function as paracrine effectors, mediating crosstalk between erythroblasts and receptor-expressing macrophages that regulates island structure. Additionally, the angiogenic factors may enable reticulocyte marrow egress by affecting endothelial cell junctional integrity.

The transcription factor GATA-1 is crucial for erythropoiesis. Absence of GATA-1 expression results in proerythroblast apoptosis [34, 35, 36, 37] and is embryonic lethal in knockout mice [38] whereas overexpression of GATA-1 blocks terminal differentiation [39, 40]. Strikingly, the presence of normally expressing cells enables GATA-1 overexpressing cells to complete normal terminal differentiation. Using a mouse model in which half of the mouse red cells are null for GATA-1 and half overexpress GATA-1 it has been observed that normal late stage erythroblasts produce a signal, termed REDS (red cell differentiation signal) that corrects the flaw(s) in GATA-1 overexpressing cells [41*]. Mechanistically this appears to involve cell-cell interaction rather than a soluble factor, underscoring the importance of intercellular signaling between erythroblasts in regulating GATA-1 activity and gene expression.

CENTRAL MACROPHAGE FUNCTION

What key functions do central macrophages perform in addition to providing adhesive interactions? It has long been recognized that they phagocytose extruded erythroblast nuclei at the conclusion of terminal differentiation [42, 43, 44]. In the fetal liver expelled nuclei rapidly expose phosphatidylserine (PS) on their surface after extrusion and are phagocytosed by fetal liver macrophages [45**]. Engulfment of nuclei is blocked by a mutant of milk-fat-globule

EGF8 [45**], known to inhibit phagocytosis of apoptotic cells by binding surface PS [46]. Extruded nuclei contain markedly decreased ATP and increased Ca2+ [45**]. Together these perturbations could inactivate the aminophospholipid translocase and activate the scramblase, resulting in movement of PS from the inner to the outer leaflet of the lipid bilayer. Additionally, analysis of erythroblast plasma membrane protein partitioning between the reticulocyte and the expelled nucleus at the time of enucleation shows that Emp partitions predominantly to the plasma membrane surrounding the extruded nucleus [47]. This feature of protein sorting would effectively provide extruded nuclei with macrophage binding partners, thereby facilitating phagocytosis.

Macrophages also secrete cytokines, including burst-promoting activity and insulin-like growth factor-1 (IGF-1), that stimulate growth of both burst forming unit-erythroid (BFU-E) and colony forming unit –erythroid (CFU-E) [48, 49, 50]. Additionally, erythropoietin mRNA has been observed in macrophages [51] although the role that central macrophages play in providing erythroblasts either macrophage-synthesized erythropoietin or presenting erythropoietin synthesized elsewhere is still under investigation. Another important question yet to be conclusively answered is whether central macrophages serve as a source of iron for hemoglobin synthesis, as was originally proposed [5]. With the recent exciting advances in our understanding of iron transporters and iron metabolism this issue can now be addressed in a meaningful manner.

Retinoblastoma tumor suppressor (Rb) protein plays a critical role in differentiation of macrophages [52**]. This ubiquitously expressed nuclear protein functions in a cell's choice to progress through the cell cycle and divide. The first suggestion that Rb protein might crucially affect erythroblastic island function were studies showing that the Rb null phenotype is embryonic lethal and that Rb null fetuses are anemic with definitive erythroblasts maturing abnormally in fetal liver and failing to enucleate [53, 54, 55]. Rb stimulates macrophage differentiation by counteracting the inhibition of Id2 on PU.1, a transcription factor that is a

crucial regulator of macrophage differentiation [52**]. Hence, Rb null fetal livers contain far fewer mature macrophages and of particular note, Rb deficient fetal liver macrophages do not bind Rb null erythroblasts. In contrast, Rb null erythroblasts attached to normal macrophages terminally differentiate and enucleate, strongly suggesting that the defect in Rb nulls is extrinsic to the erythroblast. Additionally, chimeric mice containing both normal and Rb null cells produced normal red cells from Rb null progenitors [56]. Despite these observations, controversy exists as to whether the RB null defect is erythroblast intrinsic or extrinsic; indeed, there is some evidence that it may be both [17]. Rb null erythroblasts do not differentiate normally in vitro [53], and do not undergo cell cycle exit or enucleation normally, thus supporting an additional, intrinsic erythroblast defect [57]. Further investigations will be required to clarify these issues.

LOCALIZATION OF ISLANDS

Erythroblastic islands are distributed throughout the marrow, including regions adjacent to sinusoids through which reticulocytes enter the circulation. A tantalizing question is whether islands are mobile structures and migrate toward sinusoids. Using quantitative light and electron microscopy of rat bone marrow, the differentiation stage of erythroblasts in 40 islands adjacent to sinusoids and 40 islands nonadjacent has been compared [58]. Islands in all locations contain similar numbers of basophilic and polychromatophilic erythroblasts. However, there are significantly more proerythroblasts in nonadjacent islands and significantly more acidophilic cells in islands neighboring sinusoids, clearly suggesting that erythroblastic islands may migrate to sinusoids as the cells within the island become more differentiated. If these observations are confirmed one could speculate that crosstalk between developing erythroblasts and the central macrophage could stimulate macrophage secretion of proteases that remodel underlying extracellular matrix and enable islands to be released from their original site of matrix attachments.

CONCLUSION

Our understanding of adhesive interactions, cellular cross talk, regulatory feedback pathways and intracellular signaling operating within erythroblastic islands is in an early stage. Current information opens up many exciting new avenues for exploration and it is hoped that before the 75th anniversary of the first description of erythroblastic islands we will have a much clearer understanding of molecular mechanisms crucial for normal terminal erythroid differentiation which occur within these remarkable microenvironmental niches.

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