

An Overview of Mechanism of Egress of RBC from Bone Marrow

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Review Article

Abstract

Hematopoietic stem cell in bone marrow committed to produce erythrocyte - the red blood cells. Once mature these cells continuously egress out into general circulation to perform their normal function in the body. Bone marrow generally separated from peripheral blood via sinusoid membrane that has small pores in its endothelial layer. RBCs egress out through specific sites (pores) from endothelial layer. During maturation process RBCs lose its nucleus and became discoid shape. That imparts the greater deformability into them so they could easily squeeze out through marrow aperture. The egress of RBCs further accelerated as a result of humoral factors (releasing factors) i.e., stress, cytokines, chemokines, erythropoietin and proteolytic enzymes like GTPases.

Keywords: Bone marrow; Sinusoid membrane; Deformability; Humoral factors.

1. Introduction

Bone marrow is considered as a pool of hematopoietic stem cells of different morphology and characteristics, which produce erythrocytes and leucocytes etc. Hematopoietic stem cells (HSCs) in bone marrow mainly resides in between the medullary bone and stromal cells [1]. The erythrocytes produced by HSCs have to come out from bone marrow to general circulation to perform their normal functions. Hematopoietic stem cells might be gets into general circulation helpful in defense mechanism [2-7]. The mechanism of egress of erythrocytes- the red blood cell from bone marrow is determined by many factors like the restraining barrier between bone marrow and sinusoid, pore size in the membrane, cell's ability to cross these pores and influence of certain releasing factors which enhance the passage of cells to pores of smaller diameter [8-11]. Egress mainly enhanced

under stress situations i.e., anxiety and exercise [12]. Depending upon concentration of RBC in general circulation, bone marrow proliferates to produce new RBCs and release them to general circulation to accommodate the requirements of the body. This is called as feedback mechanism that is completed when the old RBCs in general circulation die and worn out after completing their life span of 120 days. However, in the pathological condition like Malaria, Toxoplasmosis or in case of genetic defects the normal feedback system get disturbed by increasing granulocytes in bone marrow and decreasing erythrocytes and lymphocytes, disturbing the overall egress of RBCs [13-16] as shown in Figure 1. After parasitic infection decrease erythropoiesis lead to anemia [17] i.e., *Plasmodium*, *Trypanosoma* and *Babesia* sp., directly lead to the destruction of erythrocyte precursor and ultimately leading to reduction in total RBCs count [18].

1.1.Egress of erythrocytes through sinusoid membrane

Erythrocytes egress from hematopoietic cell to marrow sinusoid. Structure of marrow sinusoid under electron microscope reveals a trilaminar structure i.e., the adventitial cell towards marrow cord, a middle basement membrane and endothelial lining toward sinusoid containing pores [19]. Adventitial cell having the microfilaments structure provide area for deformed mature cells. Only the pores of endothelial allow the egress of RBCs. The pores of endothelial cell in the marrow sinusoid has diameter smaller than that of the cells [20-23]. Depending upon external requirement of erythrocytes, the marrow cell proliferate rapidly to produce greater number of erythrocytes which mature quickly and come out to general circulation to retain the steady state of body [24-27]. The egress of marrow cell when observed by placing the cells in a small millipore filters with diameter of 1 to 8 μm revealed that the rate of marrow

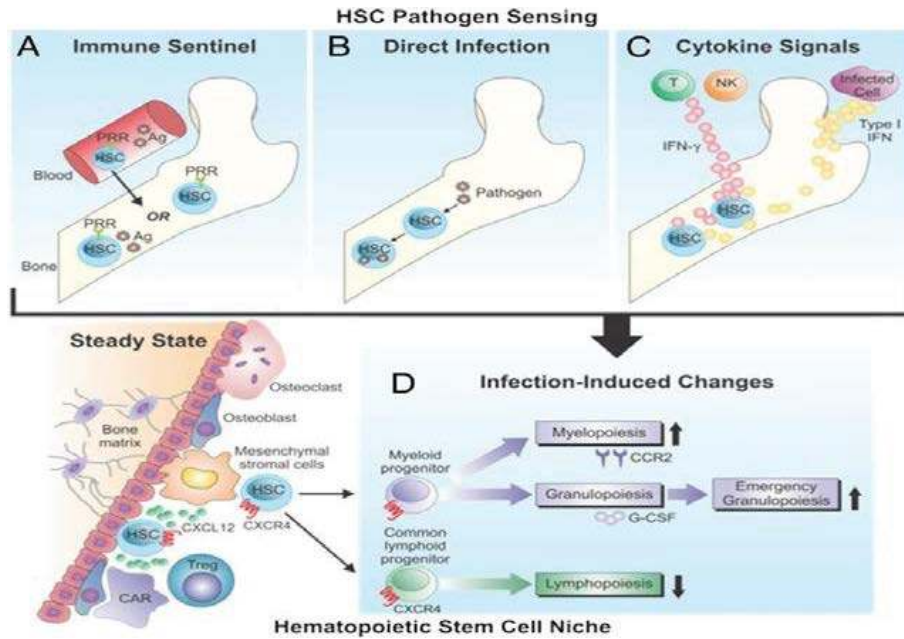


Figure 1. Response of HCS to pathogens **A)** bone marrow having pathogen recognition receptor when infected directly, the product generated in response to infection recognized while HSC in blood detected the molecular pattern of these pathogens and signals it back to bone marrow. **B)** HSC directly targeted, infected and defended in bone marrow. **C)** Pathogenic infection induced cytokines located at distal end to enter the bone marrow to initiate HSCs response within bone marrow. **D)** Level of HSCs regulated by stromal cell along with CXCL12-CXCR4 interactions which differentiate in to myeloid and lymphoid progenitors enhancing myelopoiesis, granulopoiesis and lymphopoiesis. Infection greatly enhanced myelopoiesis and granulopoiesis while decreasing lymphopoiesis.

cell egress greatly increased by increasing the pore size and by the application of chemoattractants - releasing factors. Studies indicate that only mature erythrocytes have the ability to cross the pores of barrier membrane as compared to immature cells [28] due to their deformability properties and de-nucleation [29-31]. However, some immature cells also come out into general circulation along with these mature cells [14]. The mature cells also respond rapidly to chemical attractants to come out into general circulation [8,32] (Figure 2).

1.2. RBCs egress through specific sites in marrow sinuses

The egress of reticulocytes considered first by thinning and formation of depression in the sinusoid wall after the maturation of reticulocytes [33-37].

Erythropoiesis occurs in bone marrow and at those sites' sinusoid wall penetrate and receive RBCs, moving them to central sinuses followed by marrow vein to general circulation [38]. RBCs penetrate at specific thin parajunctional sites (pores) in sinusoid wall where they encounter no resistance for crossing. This initial interaction between endothelial cells and basement membrane resulted in large number of aperture formation. These pores open only at the time of egress of RBCs while close otherwise suggesting egress of RBCs occur only at specific sites in specific time through marrow sinusoid [37].

1.3. Deformability enhance egress of RBCs

The egress of RBC is mainly determined by their shape (Discoid), presence or absence of nucleus, and properties of the membrane through which

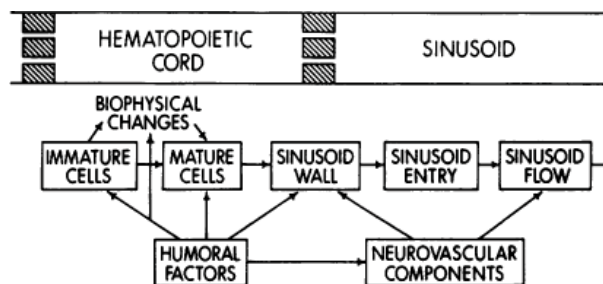


Figure 2. The egress of hematopoietic cells from hematopoietic cord to the marrow sinusoid is shown in this schematic diagram. Maturation that is stimulant for egress of cells occur in hematopoietic cord under the influence of humoral factors which greatly influence pore size of the membrane and crowding of erythrocytes [36] determined the flow of erythrocytes from bone marrow to sinusoid.

they have to pass [39]. The discoid shape and the absence of nucleus offer rapid deformability in the mature erythrocytes for their egress from bone marrow. While the immature cells cannot deform due to their shape and presence of nucleus which impart rigidity in these cells hence, they are unable to cross the sinusoid pores [28]. On the other hand, a high deposition of Ca^{2+} and Mg^{2+} in membrane makes the membrane rigid so the egress is no longer possible [40] (Figure 3).

The detailed structure of marrow sinusoid reveals pore sizes of $3\mu\text{m}$, in membrane separating hematopoietic chord and marrow sinusoid [41-43]. Mature reticulocytes due to de-nucleation and discoid shape have the well enough deformability to allow them cross this microvasculature [44]. Studies suggested that the morphology of mature reticulocytes offer them greater deformability to pass through the micropipette use in laboratory which has the pores similar to that of marrow sinusoid [45,46]. However, the pathological conditions like hemolysis and shift reticulocytes resulted in rigid premature stages of erythrocytes to egress out into general circulation [28].

2. Stimulants and egress of RBCs

In certain pathological conditions when certain stimuli are injected like Vit B12 (anemia), Haematinics (haemorrhage), cobalt (Defects in pulmonary diffusion) and iron (iron deficiency anemia), the rate of production of reticulocytes increase exponentially irrespective of the concentration of RBCs in general circulation [47]. While the administration of Sodium carbonate and adrenaline (air encephalography) resulted in increased release of erythrocytes

depleting all storage reservoirs of RBCs in bone marrow.

Administration of erythropoietin, adrenocorticotrophic hormone cytotoxic drugs along with stress, bleeding and intense physical exercise significantly increase egress of RBCs [48-51].

2.1. Effect of stress on RBCs egress

Stress stimulate the release of certain chemicals, cytokines and the proteolytic enzymes that modulate intrinsic mechanisms inducing the motility and egress of RBCs from bone marrow's specific sites [52,53] demonstrate that elevated stress conditions i.e. acute inflammation resulted in increased activity of the Hematopoietic cells to proliferate and produce greater number of RBCs permitting them to pass through sinusoid aperture [4].

2.2. Effect of internal signaling-GTPases on egress of RBCs

Guanosine triphosphatases is an internal signaling enzyme that modulates the active process of hematopoietic cell proliferation and egress [54]. Different chemokines and cytokines i.e. SDF-1/CXCR4 gets activated by the GTPase signaling [55]. CXCL12 a cytokine important for maintaining HSCs population in bone marrow, disturbance in interaction between CXCL12-CXCR4 leading to premature HSCs release from bone marrow [56,57]. Cdc42, Rho A, Rac1 and Rac2 respond strongly towards GTPase based chemokines and cytokines signals to accelerate the process of RBCs egress [58-61]. Stomatal cell derived Factor (SDF) a cytokine along with mobilization of RBC has important roles in retention, and survival. Various bone marrow stromal

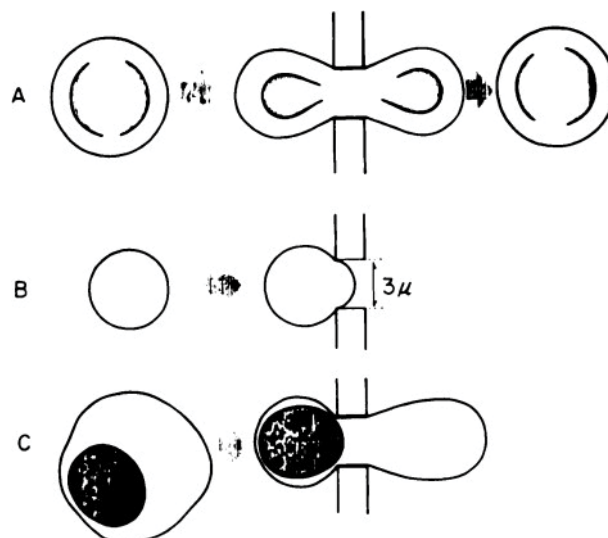


Figure 3. Diagram represent the egress of RBCs depend upon presence or absence of nucleus and deformability. A) The passage of normal discoid RBCs from membrane aperture of $3\mu\text{m}$ due to their greater deformability. B) rigidity prevents the passage of spherocyte because they are unable to deform. C) Immature RBCs having nucleus unable to pass through pores.

niche cells i.e., endosteal bone lining osteoblasts [62] and bone marrow endothelium [63] express the SDF. Recently hypothesized that administration of AMD3100 interrupts SDF stimulation that led to RBC retention.

2.3. Effect of cytokines and chemokines on RBCs egress

Granulocyte colony stimulating factor (G-CSF)—a cytokine and most widely used hematopoietic egress inducing agent [64]. And the excessive repeated administration of G-CSF enhances mobilization of bone marrow contents into general circulation [65]. G-CSF gets activated by adrenergic stimulation [66]. Administration of AMD3100—a chemokines either singly or in combination with G-CSF enhance the mobilization of HSCs [67-70] reported that the AMD300 cause diminishing SDF-1/CXCR4 (fucoidan and catecholamine) interaction in bone marrow

causing their elevated concentration of SDF-1 in peripheral blood. This loosen interaction between SDF-1/CXCR4 facilitates increase erythrocyte egress from bone marrow [71-73]. Similarly, another chemokine T-140 enhance egress of hematopoietic cells from bone marrow [74,75] reported that in contrast to G-CSF cytokine which requires daily administration the cytokines like CXCR2 ligand GROβ by secreting certain wall digesting proteolytic enzymes enable egress within 20 minutes. The whole process of RBCs egress under the influence of different external and internal stimulants shown in Figure 4.

Under normal condition hematopoietic cells product (reticulocytes) retained in bone marrow in retention condition and only a small fraction of cell is freely circulating in general circulation. SDF-1 in bone marrow permit them to retain there while under stress condition level of G-CSF along with

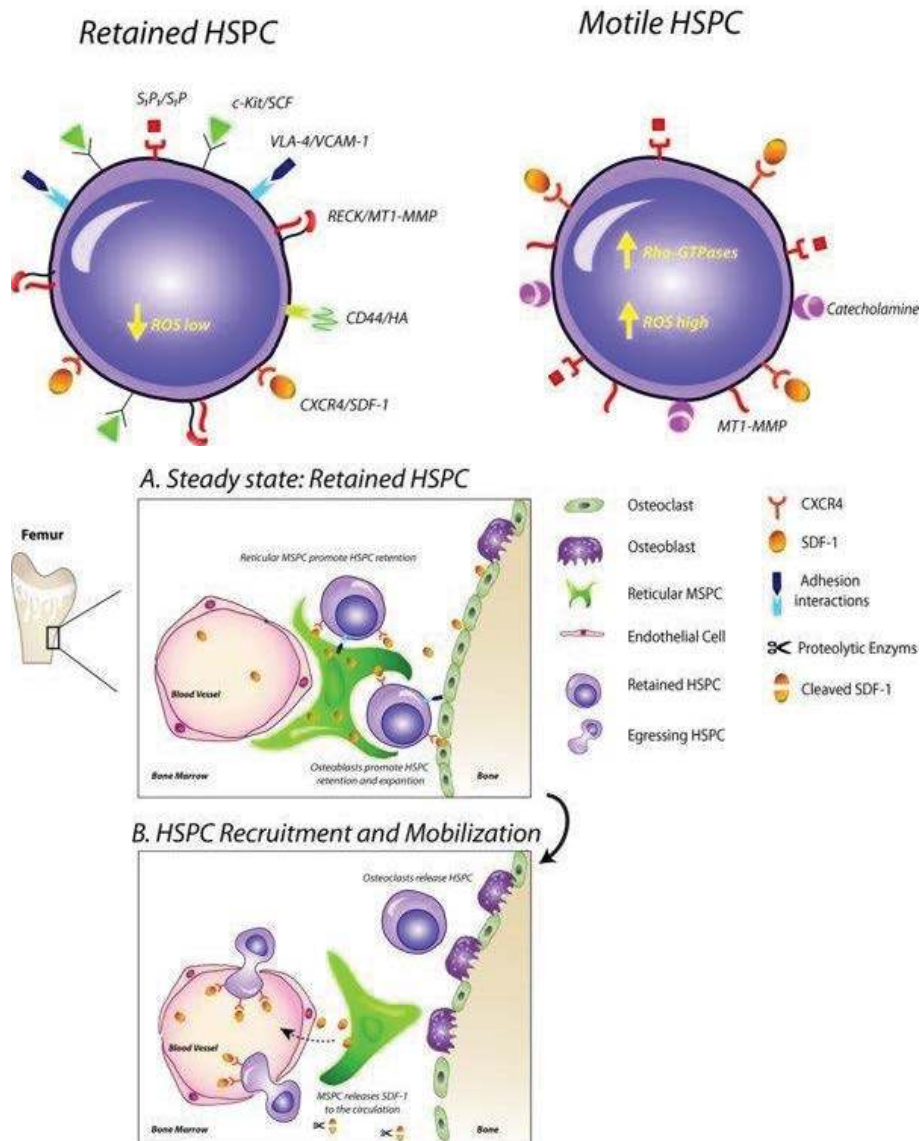


Figure 4. Retention and Egress of RBCs from Bone marrow.

AMD3100 increased which greatly accelerate the process of egress. Different humoral factors act on the membrane surface to greatly enhance the egress mechanism [52].

2.4. Erythropoietin, bone remodeling and egress of RBCs

Endogenous and exogenous erythropoietin administration resulted in increased egress of RBCs by increasing the number of pores in sinusoid membrane, decreasing the cells of adventitial layer and diminution of hematocrit cells. But the excessive administration of erythropoietin counteracts this effect due to increase hematocrit level [76,77]. Osteoblasts (by MSPC) and osteoclasts (by monocyte) established bone equilibrium within endosteum in the vicinity of HSCs [78]. Bone formation and degeneration disturb the stem cells niches and ultimately affect the HSCs balance in bone marrow. Administration of Granulocyte colony stimulating factor (G-CSF) or cyclophosphamide injections altered the morphology of osteoblasts by expanding it and promoting HSCs proliferation, resulting in reduced transcription of SDF-1, and VCAM-1 thus HSCs lose their retention [79-83].

3. Conclusion

Overall, it is concluded from the review of literature that the maturation process enhances the deformability of normal discoid shape erythrocytes. This deformability permits them to easily pass through the pores in sinusoid membrane. The hematopoietic cells respond strongly to the certain humoral factor that increases their permeability through the membrane by increasing pore size. But the onset of infection from pathogen and parasites disturb the dynamics of HSCs and ultimately reduce the RBC egress. Certain chemokines and cytokines i.e., G-CSF, AMD3100, CXCR12 and SDF deliberately enhance RBC egress from bone marrow. CXCR2 ligand GRO β by secreting certain wall digesting proteolytic enzymes enable egress within 20 minutes.

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