Comment on Toda et al, page 3963

**Red cell island dances: switching hands**

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In this issue of *Blood*, Toda et al present a shift in the paradigm of erythroid enucleation and provide novel tools to further study and optimize terminal erythroid maturation in vitro.\(^1\)

We have known for more than 50 years that red blood cells (RBCs) are generated in bone marrow erythroblastic islands, where erythroid precursors in various stages of maturation are bound to a central macrophage.\(^2\) Erythroblastic islands have been proposed to facilitate feedback from more mature erythroid cells to limit the expansion of younger cells, to allow macrophage signaling and nutritional support, and to eliminate the nuclear waste resulting from the synthesis of enucleated reticulocytes.\(^3\) The most striking visual aspect of this erythropoietic maturation is the last step, when the orthochromatic erythroblast goes through remarkable undulations, like a pot coming to boil, with the bulk of its cytoplasm pulling away from the nucleus until it divides into 2 very different cells.\(^1,4\) The enucleated reticulocyte continues to mature into the functional RBC. The other cell, a pyrenocyte containing the condensed nucleus surrounded by a thin layer of cytoplasm, signals the macrophage that it is the half to be eaten by exposing phosphatidylserine on its cellular membrane.\(^5\) Although erythropoiesis can be recapitulated in vitro, erythroblasts often stumble over this last step with inefficient enucleation, an issue that has challenged the ex vivo generation of RBCs.\(^6\)

Adhesion between erythroid cells and macrophages involves integrin $\alpha_4\beta_1$ and vascular cell adhesion molecule (VCAM), respectively, and interruption of VCAM interactions disrupts erythroblast binding to macrophages.\(^7\) During enucleation, integrin $\alpha_4\beta_1$ is asymmetrically apportioned to the pyrenocyte.\(^8\) Therefore, in a simple model, the pyrenocyte retains integrin $\alpha_4\beta_1$, causing it to remain behind to be eaten, whereas the reticulocyte loses integrins and can leave the island. However, Toda et al show that the simplest model is not always correct. They confirm that erythroblasts bind VCAM and that reticulocytes do not, but surprisingly, pyrenocytes also do not bind VCAM because their integrin $\alpha_4\beta_1$ is in an inactive form. Instead, borrowing steps from apoptosis, Toda et al find that MerTK receptors on island macrophages bind phosphatidylserines on pyrenocyte membranes using Protein S as a linker. MerTK is a member of the TAM family of receptors that are involved in recognition and engulfment of apoptotic cells and that use specific proteins, such as Protein S for MerTK, as a linker. MerTK is a member of the TAM family of receptors that are involved in recognition and engulfment of apoptotic cells and that use specific proteins, such as Protein S for MerTK, as a linker between phosphatidylserine and themselves.\(^9\)

Furthermore, the authors make the important observation that increasing the viscosity of the media during ex vivo island maturation by the addition of 1% methylcellulose increased the rates of pyrenocyte consumption by macrophages. They hypothesize there is decreased diffusion of pyrenocytes during the switch from VCAM- to MerTK-based binding that may more closely emulate what occurs in the closely packed bone marrow microenvironment. Thus, a new model of terminal erythropoiesis emerges (see figure) in which the erythroblast lets go...

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**Model of interactions in erythroblastic islands.** Erythroblasts bind macrophages via integrin $\alpha_4\beta_1$ and VCAM interactions until they reach the stage of enucleation, at which time the erythroblast separates into a released enucleate reticulocyte and nucleated pyrenocyte. The pyrenocyte then exposes phosphatidylserine on its surface, which binds to Protein S and stimulates the MerTK receptor on the macrophage, causing its phagocytosis. See Figure 5 in the article by Toda et al that begins on page 3963.
of its partner macrophage as it divides into a released reticulocyte and pyrenocyte, but the latter is caught by the macrophage and phagocytosed using mechanisms shared with apoptosis.

Along with facilitating the capture of pyrenocytes by macrophages, Toda et al observed increased enucleation on the islands when methylcellulose was added to the cultures. Under these conditions, enucleating cells were identified by their asymmetric rippling in time-lapse videos, which were followed by release of the reticulocyte. However, in typical liquid media, these rippling cells leave the island without completing enucleation. It is known that in vitro erythroid enucleation is facilitated by shear stress such as pipetting, and the authors suggest that higher viscosity media may increase shear stress generated by dynamic ruffling of the macrophages. Alternatively, increased viscosity may enhance the effectiveness of the highly active movements of the presumptive reticulocyte as it attempts to separate from the nucleus. Increased macrophage attachment may also provide a counterforce to push against. It was not reported if increased viscosity assisted erythroblast enucleation even without macrophage involvement. Whether it is macrophage fanning, reticulate struggling, or a tug of war over the pyrenocyte, this improvement of ex vivo erythroid maturation reported by Toda et al offers us new models and techniques to study erythroid enucleation.

As with any good paradigm shift, this work raises many questions for future research. One important consideration is that the authors report normal bone marrow and spleen erythropoiesis in MerTK-disrupted mice, implicating additional players. The authors identify another TAM family member, Axl, and its Gas6 linker as possible candidates to bind pyrenocytes. All of this work was performed with splenic erythroblastic islands under anemic conditions, and it remains to be determined if this process is differently regulated during steady-state conditions. Indeed, it would be interesting to know if the MerTK-disrupted mice have normal responses to erythropoietic stress. Additionally, other mediators of erythroid macrophage binding such as intercellular adhesion molecule and erythroid macrophage protein may continue to anchor the pyrenocytes during terminal maturation.3

Perhaps the most remarkable aspect of in vivo erythroblastic islands is their productiveness, producing more than 2 million new reticulocytes every second in adult humans.10 Although we can replicate erythroid maturation with erythroid cells alone, a better understanding of macrophage–erythroid interactions may help us recapitulate the island’s efficiency making the ex vivo production of units of blood that contain trillions of RBCs a practical reality.

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REFERENCES

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CLINICAL TRIALS & OBSERVATIONS

Comment on Bachanova et al, page 3855

Can Treg elimination enhance NK cell therapy for AML?

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In this issue of Blood, Bachanova et al describe how modulation of the inhibitory tumor environment may enhance natural killer (NK) cell clinical activity and produce encouraging results in the treatment of refractory acute myeloid leukemia (AML).1 NK cells are highly proliferative, early responders of the innate immune response that in preclinical models can exert potent activity against a wide range of malignancies, including AML, and across HLA barriers. Considerable efforts have been made to exploit this activity in clinical trials but with only modest success.

NK cells are activated by inflammatory cytokines, usually produced in response to pathogens. Following activation, NK cells upregulate activating and inhibitory receptors including killer immunoglobulin-like receptors (KIRs) that recognize HLA class I molecules. The highly polymorphic nature of both HLA and KIR molecules determines whether the NK cell subsequently ignores a target cell or kills it and proliferates. Because KIR receptors are inhibited by autologous HLA class C molecules, many clinical investigators have chosen to infuse allogeneic NK cells to ensure that they can kill recipient tumor cells.2–4

Several clinical trials have revealed the potential of NK cells as an immunotherapy, but barriers to their wider efficacy have included both their tolerance to self-major histocompatibility complex molecules and their susceptibility to immunosuppressive elements that are present at the sites of many tumors. These elements include inhibitory cytokines, myeloid-derived suppressor cells, and T-regulatory cells (Tregs). Although the range of immunosuppressive elements within tumors is daunting, most converge on and inhibit signaling pathways that are also targets of activating ligands, so that shifting the balance in favor of

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