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REVIEW

Development of autologous blood cell therapies

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Allogeneic hematopoietic stem cell transplantation and blood cell transfusions are performed commonly in patients with a variety of blood disorders. Unfortunately, these donor-derived cell therapies are constrained due to limited supplies, infectious risk factors, a lack of appropriately matched donors, and the risk of immunologic complications from such products. The use of autologous cell therapies has been proposed to overcome these shortcomings. One can derive such therapies directly from hematopoietic stem and progenitor cells of individuals, which can then be manipulated ex vivo to produce the desired modifications or differentiated to produce a particular target population. Alternatively, pluripotent stem cells, which have a theoretically unlimited self-renewal capacity and an ability to differentiate into any desired cell type, can be used as an autologous starting source for such manipulation and differentiation approaches. Such cell products can also be used as a delivery vehicle for therapeutics. In this review, we highlight recent advances and discuss ongoing challenges for the in vitro generation of autologous hematopoietic cells that can be used for cell therapy.

In a number of blood disorders, hematopoietic stem cell (HSC) transplantation is currently the only available curative therapy. Approximately 50,000 HSC transplantation procedures are performed each year around the world [1]. However, HSC transplantation is generally not the first line of treatment for the majority of blood disorders. This is largely attributable to the numerous complications that can result from obtaining and transplanting HSCs from a donor (termed allogeneic transplantation) [1,2]. For example, an appropriate match does not exist for the majority of patients with blood disorders and, even when a matched donor is identified, significant morbidity due to immunologic incompatibility between recipient and donor remains common [1,2]. An emerging alternative is the possibility of autologous transplantation, in which HSCs or other cell sources derived from an individual are used as material for hematopoietic reconstitution of that individual, which can thereby avoid many of the complications inherent to allogeneic transplantation. Typically, in autologous transplantation, stem cells are harvested and modulated ex vivo, the individual is subsequently conditioned with chemotherapy, and the harvested cells are transplanted to reconstitute the hematopoietic system. Unfortunately, this approach currently is associated with significant limitations because an individual's HSCs often harbor the precise mutations that caused the blood disorder, particularly in genetic and malignant blood diseases [3].

In many blood disorders, including sickle cell disease, thalassemia, bone marrow failure syndromes, and other chronic cytopenias, transfusion of specific blood cell components such as red blood cells (RBCs) or platelets is sufficient to confer clinical benefit [4,5]. Despite the prevalence of this procedure and the ready availability of donor-derived blood products, there are limitations, including inadequate blood supply in certain circumstances and transfusion-associated risks [6]. In addition, patients who receive blood transfusions frequently are at risk of developing alloimmunization, which presents a significant challenge in obtaining appropriately matched blood products [7]. Therefore, the development of alternative or complementary approaches is under active investigation.

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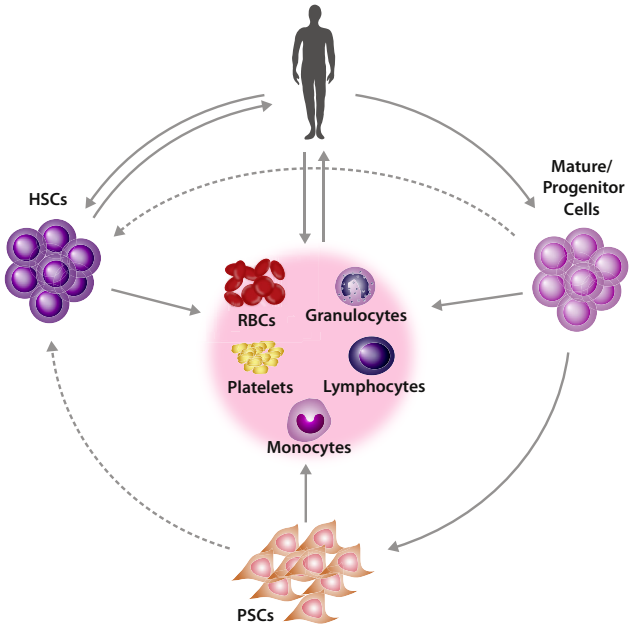

Figure 1. Strategies for producing and applying autologous blood cell therapies. Autologous transplantation and transfusion cell sources can begin with the isolation of mature cells or progenitors such as those found in peripheral blood mononuclear cells. Such mononuclear cells can either be differentiated directly toward mature blood cells or reprogrammed directly into the desired cell types, including HSCs. PSCs can be differentiated into downstream mature blood cells including RBCs, platelets, monocytes, granulocytes, and lymphocytes and, potentially, into HSCs for autologous transplantation. Solid lines represent approaches that can currently be performed robustly; dashed lines represent approaches that are currently being developed or that will require further refinement.

One promising candidate approach is the generation of blood cells in vitro. In the case of blood cell transfusions, immature hematopoietic progenitors can be collected from a patient, expanded ex vivo, terminally differentiated (e.g., into RBCs), and ultimately transfused back into the same patient (Fig. 1). Moreover, the ability to obtain large numbers of HSCs from patient-derived pluripotent stem cells (PSCs) or other autologous sources for use in hematopoietic transplantation is a major goal for the field of regenerative medicine. In this review, we discuss recent work that may advance the availability and clinical utilization of autologous blood cell therapies while also highlighting challenges ahead to make the use of such therapies clinically feasible.

The generation of blood cells ex vivo from hematopoietic stem and progenitor cells

Hematopoiesis maintains the steady-state level of blood cells and this process is regulated by a combination of various cytokines that direct self-renewal of stem cells and differentiation of progenitors [8,9]. Based on extensive studies of hematopoietic differentiation in vivo, this process has been recapitulated in vitro to produce multiple mature blood cell lineages, including RBCs, platelets, and neutrophils (Table 1) [61]. For example, the addition of erythropoietin leads preferentially to erythroid-lineage commitment for RBC production, whereas the addition of thrombopoietin results preferentially in megakaryocyte-lineage differentiation for platelet production [61]. Importantly, some in vitro–produced blood cells can circulate when transfused into human recipients. For example, a proof-of-principle experiment showed that in vitro-cultured RBCs could be used successfully for autologous transfusion and the transfused RBCs were maintained in the circulation of a recipient [10]. Although the differentiation of downstream hematopoietic cells has been well studied, a major challenge that remains to make such therapies clinically useful is the need to scale production in a cost-effective manner.

A number of groups have developed culture systems that allow for the extended proliferation of lineage-restricted progenitors by perturbing regulatory genes (Table 1). For example, it has been shown that perturbing one or a combination of regulatory factors, including *MYC*, *BCL-XL*, *HPV16-E6/E7*, *SOX2*, *TP53*, and *Bmi1*, allows the erythroblasts to proliferate stably for a few months while still retaining the ability to mature terminally to some extent [11–13,20]. Therefore, progenitors or immature precursors are expanded as much as possible before initiating terminal maturation. One limitation is that, in most of these cases, the differentiation observed from such cells does not mimic the efficient terminal maturation seen with unperturbed primary hematopoietic progenitors. In addition to these methods, it has been shown that the suppression of *SH2B3*, a negative regulator of hematopoietic cytokine signaling, can increase the production of RBCs derived from human hematopoietic stem and progenitor cells (HSPCs) significantly [14]. In contrast to the generation of immortalized cell lines, the method using *SH2B3* suppression in HSPCs increases the yield of RBC production while not perturbing and actually improving overall differentiation [14]. Given these significant advances in our understanding of the mechanisms governing erythroid self-renewal and differentiation, one logical next step would be to screen small molecules that can activate or inhibit those regulatory factors or other molecular pathways to establish long-term proliferating erythroblasts in vitro for future clinical use. Because RBCs have an average circulating lifespan of 120 days and are enucleate, the generation of RBCs in vitro would also allow for their use as vehicles to deliver various molecules such as therapeutics that have poor bioavailability or that need to be targeted to a particular tissue [62]. For example, it has been shown as a proof-of-principle that mouse RBCs can be engineered to express modified surface proteins that enable targeting to particular tissues and delivery of molecules via these engineered RBCs [63].

Recent advances have also allowed us to move toward improving upon and making large-scale production of platelets for autologous transfusion an achievable goal