Successful ex vivo expansion of mouse hematopoietic stem cells

Hideo Ema*

State Key Laboratory of Experimental Hematology, National Clinical Research Center for Hematological Disorders, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China

Abstract

Ex vivo expansion of hematopoietic stem cells (HSCs) is considered the holy grail in stem cell biology and therapy, as it has long been difficult to make this procedure possible. Yamazaki's research team has established new, polyvinyl alcohol-based culture conditions and shown a significant expansion of mouse HSCs from a small number of cells after a month of culture. Surprisingly, expanded HSCs were able to reconstitute unconditioned normal mice. There is generally a technical concern in limiting dilution assay to estimate a fold-expansion of HSCs. But, this work paves the way toward expansion of human HSCs useful for transplantation medicine.

Keywords: Ex vivo expansion, Hematopoietic stem cells, Polyvinylalcohol, Self-renewal

Hematopoietic stem cells (HSCs) have been used for stem cell transplantation to treat patients with leukemia and other diseases for several decades.[1] However, HLA-matched donors are often unavailable, and the proportion of HSCs in donor cell sources is limited. Thus, methods enabling ex vivo expansion of HSCs have long been sought. HSCs easily differentiate in vitro, and it is difficult to induce their self-renewal while preventing differentiation. A number of culture systems designed to expand mouse HSCs in vitro have been reported.[2] However, it is sometimes difficult to reproduce published data, mostly because culture conditions are unstable, and in vivo assays used to determine the number of expanded HSCs are not precise.

Yamazaki et al. performed a series of experiments in an effort to establish stable and reproducible culture conditions.[3–5] First they found that recombinant albumin can replace bovine serum albumin (BSA) in serum-free culture of HSCs.[3] They then extensively optimized serum-free culture conditions,[5] and finally showed that polyvinyl alcohol (PVA) can replace recombinant albumin.[4]

Several decades ago PVA was suggested to serve as a replacement for BSA in embryology experiments,[6] but investigators have continued to use BSA in many types of tissue cultures. Now, Yamazaki's team has tested PVA and found it superior to albumin for use in HSC expansion.[4] Their serum-free and albumin-free media consists of 0.1% PVA in Ham's F-12 medium with a mixture of insulin, transferrin, selenium, and ethanolamine from Gibco. It was previously known that HSCs efficiently divide in the presence of stem cell factor (SCF) and thrombopoietin (TPO).[7] Yamazaki's team retitrated concentrations of these cytokines and reported that in their system a combination of 10 ng/mL SCF and 100 ng/mL TPO produced optimal expansion.[4] They also reported that HSCs undergo more efficient expansion in fibronectin-coated dishes.[4]

Competitive repopulation has been used as the most reliable assay to detect HSC activity. For such analysis, test donor and competitor cells are usually cotransplanted into myeloablated mice. Researchers have recently become aware that competitive repopulation can be undertaken in normal mice also.[8–10] In that case, endogenous HSCs in the host mouse simply complete with transplanted test donor-derived HSCs. The percent chimerism in peripheral blood is reflected by the ratio of the number of donor HSCs to the number of host HSCs.

In vivo limiting dilution type assays have been used to measure the number of HSCs in culture, as the frequency of HSCs in cultured cells is usually very low. A large number of samples at three or more appropriate cell doses should be analyzed to determine HSC frequency with reliability.[11] However, a very small number of mice per cell dose are often used for practical reasons, making it difficult to estimate HSC frequency with precision. Yamasaki's team estimated HSC frequency in their culture after a month by in vivo limiting dilution and calculated the fold-increase as 236-899.[4]

Here, I have calculated the fold-increase in a different way using their data.[4] It is estimated that a C57BL/6 mouse has $\sim 4.5 \times 10^8$ bone marrow cells in its entire body.[12] Given that $CD150^+CD34^-c\text{-}Kit^+Sca\text{-}1^+Lin^-$ ($CD150^+34^-KSL$) cells account for ~0.001% bone marrow cells,[13] a mouse harbors ~4500 $CD150^+34^-KSL$ cells. After transplantation with

* Address correspondence: Hideo Ema, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 288 Nanjing Rd, Tianjin 300020, China. Phone: (86) 22 2390 9176; E-mail: hema@ihcams.ac.cn (H. Ema).

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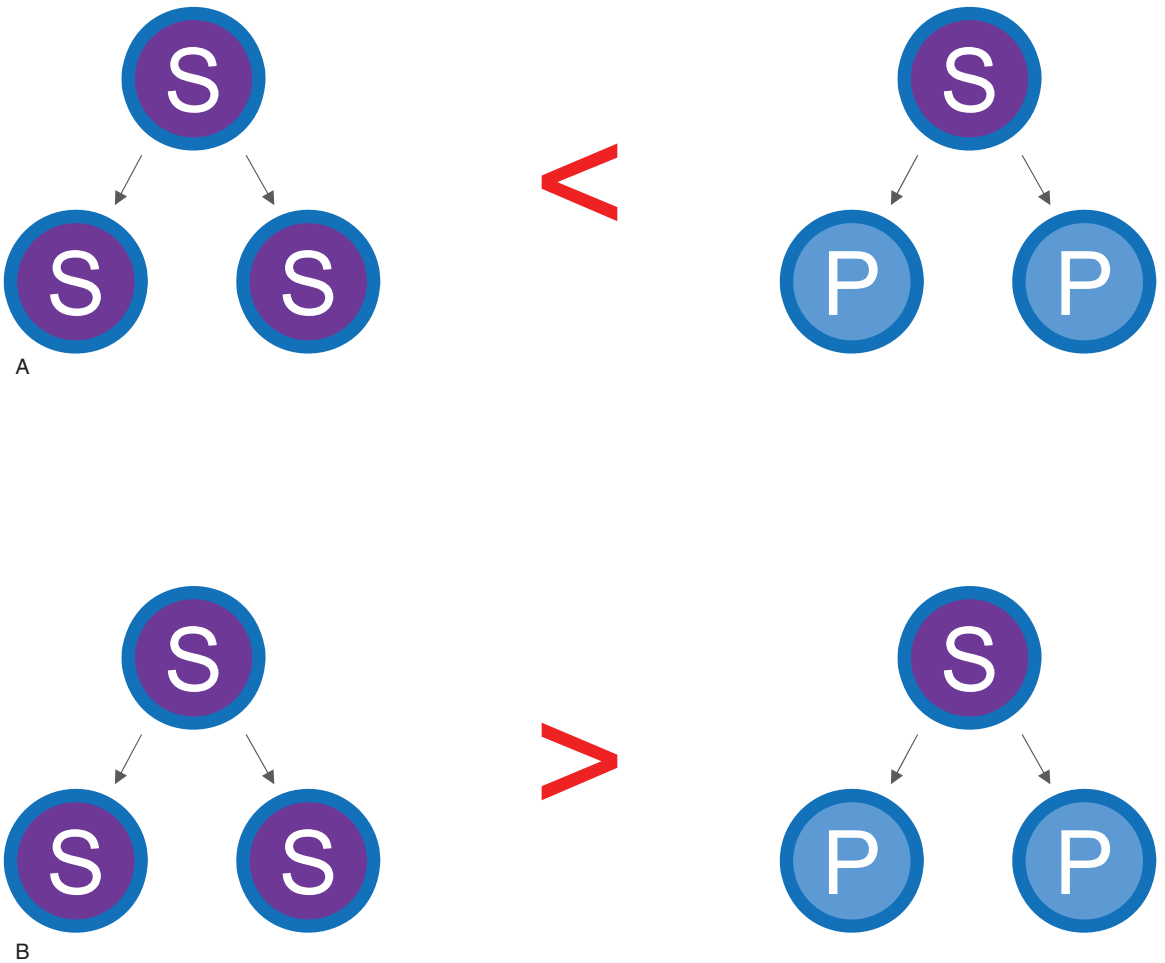

Figure 1. Symmetrical HSC self-renewal takes place in the presence of PVA. In this model, PVA increases the probability of symmetrical self-renewal of HSCs. Symmetrical differentiation: an HSC (S) gives rise to two progenitor cells (P). Symmetrical self-renewal: an HSC gives rise to two HSCs. (A) The probability of symmetrical differentiation exceeds that of symmetrical self-renewal in the presence of SCF and TPO. (B) The probability of symmetrical self-renewal exceeds that of symmetrical differentiation in the presence of SCF, TPO, and PVA.

cultured cells into normal mice, on average, 10% chimerism was obtained, as reported in Yamazaki's study.[4] Assuming that transplanted HSCs are functionally equivalent to host HSCs and all transplanted HSCs engraft, the number of HSCs (N) in cultured cells can be calculated as: $N/(4500+N)=0.1$. Thus, in this case, $N=500$. Yamazaki's team used 50 $CD150^{+}34^{-}KSL$ cells to initiate the culture. Thus, the fold-increase of $CD150^{+}34^{-}KSL$ cells in culture appeared to be 10 by my calculation. The percentage of $CD150^{+}34^{-}KSL$ cells in bone marrow varies, depending on how sorting gates, particularly the CD150 gate, are set.[13] If that gate is relatively wide, as was the one used by Yamazaki et al.,[4] the fold-increase could be up to 40. Nevertheless, their data clearly show significant ex vivo expansion of functional HSCs.

An important question to be addressed now is how PVA acts to support HSC self-renewal, as PVA may function differently from recombinant albumin. Currently, we assume that PVA reversibly antagonizes HSC differentiation, enabling more symmetrical self-renewal than symmetrical differentiation (see Fig. 1). Once we clarify the mechanism, we should be able to apply this protocol to achieve safe and efficient expansion of human HSCs expansion in the near future.

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