SPECIAL FOCUS INTRODUCTION

Thrombopoietin and the Hematopoietic Stem Cell

By Kenneth Kaushansky

The factors that contribute to the maintenance of hematopoietic stem cells and their commitment to various hematopoietic lineages have been sought for many decades. Unfortunately, our present understanding of this process is far from complete. Accumulating evidence suggests that stem factor (SF; also termed stem cell factor, mast cell growth factor, or c-kit ligand) is at least one of the proteins that contributes to the maintenance of normal stem cell numbers. Its genetic elimination in mice, or that of its receptor c-kit, leads to a profound reduction in the number of hematopoietic stem cells, assayed as marrow cells that can permanently repopulate all of hematopoiesis in a lethally irradiated recipient. In addition, the administration of SF to mice expands the number of transplantable hematopoietic stem cells. Despite these results, SF alone cannot maintain the numbers of stem cells in vitro, suggesting that these in vivo effects are due to the interaction of SF with other cytokines. Two proteins thought to play such a role are FLT-3 ligand (FL) and interleukin-11 (IL-11). Genetic elimination of the FLT3 receptor leads to a fivefold reduction in the number of stem cells. In contrast to the SF/c-kit and FL/FLT3 receptor systems, genetic elimination of the IL-11 receptor fails to affect hematopoietic stem cell numbers; rather, the evidence that IL-11 affects stem cells derives almost entirely from in vitro expansion experiments. Neither SF, FL, or their combination are sufficient for maintaining the number of hematopoietic stem cells in serum-free culture. However, the addition of IL-11 to either SF or FL maintains, and the presence of all three cytokines modestly expands the number of transplantable stem cells in serum-free ex vivo cultures. Several other cytokines have been tested for their capacity to maintain or expand the number of hematopoietic stem cells, including IL-1, IL-3, IL-6, IL-12, and the blockade of transforming growth factor-β (TGF-β), but none have proven essential by the rigorous criteria noted above, genetic elimination reducing, or forced expression expanding the number of marrow cells capable of long-term hematopoietic repopulation. Thus, it is of great interest when another hematopoietic cytokine is reported to directly affect the hematopoietic stem cell.

The recent cloning of thrombopoietin and characterization of the recombinant protein fulfilled its predicted role as the primary regulator of megakaryocyte and platelet development. Alone, thrombopoietin stimulates megakaryocyte colony growth from marrow progenitors, stimulates the maturation of immature megakaryocytes, and supports the formation of functional platelets. However, soon after the recombinant protein became available, evidence began to emerge that thrombopoietin plays a wider role in hematopoiesis than initially anticipated, exerting profound effects on primitive hematopoietic cells. A diverse range of studies have supported this concept, including studies of thrombopoietin receptor expression and function, the administration of the hormone to animals, genetic elimination of thrombopoietin or its receptor, and single cell in vitro tracking experiments.

The thrombopoietin receptor (c-mpl) was first recognized as the transforming oncogene (v-mpl) of the murine myeloproliferative leukemia virus, an agent that induces not only megakaryocytic hyperplasia but a pan-myeloid disorder. Consistent with this effect, the normal receptor is expressed not only on megakaryocytes and platelets, but also on a large fraction of CD34+ cells, from which thrombopoietin enhances megakaryocyte formation. Administration of thrombopoietin to normal animals expands the number of marrow and splenic progenitors of all hematopoietic lineages, and accelerates their recovery if given following myelosuppressive therapy. Furthermore, in addition to the profound thrombocytopenia and reduced marrow levels of megakaryocytes and their progenitors, mice carrying targeted deletions of the genes for thrombopoietin or its receptor were found to possess greatly reduced numbers of progenitors committed to the erythroid and myeloid lineages. However, it was unclear whether these findings were the result of thrombopoietin affecting the committed hematopoietic progenitor, or due to a direct effect on cells at an earlier stage of development.

While these in vivo experiments were being conducted, the direct effects of thrombopoietin on candidate populations of stem cells were also being tested. Ku et al found that adding thrombopoietin to either IL-3 or SF speeds entry of post-SFU/lin-/ly6+/kit+ murine cells into the cell cycle, and greatly augments the development of progenitor cells committed to all hematopoietic lineages. Sitnicka et al arrived at the same conclusions using an extremely enriched population of hematopoietic progenitors.

From the Division of Hematology, University of Washington, Seattle. Address reprint requests to Kenneth Kaushansky, MD, Division of Hematology, Box 357710, University of Washington, Seattle, WA 98195. © 1998 by The American Society of Hematology. 0006-4971/98/9201-0045$3.00/0
poietic stem cells. As these latter experiments were conducted in single cell cultures, thrombopoietin appeared to exert a direct effect on primitive hematopoietic cells, at least in vitro.

Two more recent studies directly address this question. Kimura et al. showed that targeted disruption of the mpl gene greatly reduces the number of CFU-S,S_{A,B}, and the marrow cells of the nullizygous mice were markedly inferior to those derived from wild-type littermates in a competitive repopulation assay of stem cell activity. And in this issue of Blood, Solar et al. present three lines of evidence that firmly establish an important role for thrombopoietin in stem cell physiology. Using an mpl-specific antibody to divide murine AA4+/Sca+/kit+ fetal liver cells or lin+/Sca+/kit+ adult marrow cells into mpl+ and mpl− fractions, these investigators demonstrated that essentially all of the hematopoietic repopulating activity (assessed at 24 weeks) resides in the mpl+ subfraction. Next, human marrow CD34+/CD38−/mpl+ cells were shown to engraft SCID-hu bone mice far more efficiently than CD34+/CD38−/mpl− cells. Finally, Solar et al confirmed and extended the work of Kimura et al, quantifying the engraftment defect displayed by marrow cells from mpl nullizygous mice, a deficiency quite similar in magnitude (sevenfold reduction in repopulating units) to that found in FLT3- and SF-deficient mice.

Thus, as thrombopoietin is the sole ligand for the mpl receptor, it is now firmly and directly established that the hormone affects hematopoietic stem cells. There appear to be at least three immediate implications of this conclusion. First, the administration of thrombopoietin may evoke a far greater effect on hematopoietic recovery than initially anticipated. This prediction has been substantiated, at least in many preclinical trials of the agent, and it would seem prudent that clinical trials should be designed with this possibility in mind. Second, thrombopoietin could provide an important adjunct in attempts to expand the numbers of hematopoietic stem cells for clinical use. The use of umbilical cord blood cells for transplantation and stem cells of several origins in gene therapy protocols have provided an important impetus to expand primitive hematopoietic cells. Evidence of the capacity of thrombopoietin to augment stem cell expansion is accumulating; the hormone is the most potent single agent at expanding long-term culture initiating cells in serum-free culture, a surrogate assay for the human hematopoietic stem cell, and the combination of FL plus thrombopoietin greatly expands the output of these cells in long-term umbilical cord blood cell cultures. Moreover, agents such as thrombopoietin, which accelerate stem cell entry into the cell cycle, are particularly attractive for the expansion of stem cells used for gene therapy, where target cell proliferation is required for successful retroviral vector integration. Third, the ability of thrombopoietin to support the survival and proliferation of hematopoietic stem cells may also herald adverse effects if the hormone is used in patients with myeloproliferative disorders. Similar warnings were sounded with the use of GM-CSF and G-CSF after therapy for acute myeloid leukemia, concerns that have not been realized. However, G- and GM-CSF do not affect the hematopoietic stem cell, the likely cellular origin of most cases of myeloproliferative disease. Thus, once again, we need to carefully monitor the administration of thrombopoietin to such patients.

Our understanding of stem cell biology has advanced with the demonstration that thrombopoietin is one of the factors that supports the survival and proliferation of these intriguing cells. However, many questions remain unanswered. The nature of the intracellular signals initiated by thrombopoietin and the other proteins that affect stem cell development are unknown, as are how these events interact with the developmental programs initiated by transcription factors such as SCL/TAL1, Rbtn-2, or GATA-2 to affect hematopoietic stem cell expansion and lineage determination. It is now up to basic scientists and clinical investigators to advance our understanding of this process and to exploit these effects for therapeutic benefit.

REFERENCES

8. Peters SO, Kittler ELW, Ramshaw HS, Quesenberry PF: Ex vivo expansion of murine marrow cells with interleukin (IL)-3, IL-6, IL-11 and stem cell factor leads to impaired engraftment in irradiated hosts. Blood 87:30, 1996
9. Van der Loo JCM, Ploemacher RE: Marrow and spleen-seeding efficiencies of all murine hematopoietic stem cells are decreased by preincubation with hematopoietic growth factors. Blood 85:2598, 1995


Thrombopoietin and the Hematopoietic Stem Cell

Kenneth Kaushansky