Blood Science

Quickly attainable and highly engrafting hematopoietic stem cells

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There are three clinically used sources of hematopoietic stem cells (HSC) and hematopoietic progenitor cells (HPC) for hematopoietic cell transplantation (HCT): bone marrow (BM), umbilical cord blood (CB), and cytokine-induced mobilized peripheral blood (mPB) cells.[1–5] Each source of clinically used cells has its advantages and disadvantages. Of these, mPB cells make up the majority of autologous and allogeneic HCT efforts worldwide. The advantage of mPB is that one can, in many cases, usually collect more than enough cells to ensure a rapid and long-lasting donor graft, be it for autologous or allogeneic HCT. The "gold" standard for collection of mPB has been, and continues to be, the cells mobilized from the BM to peripheral blood by multiple additions of the cytokine granulocyte colony-stimulating factor (G-CSF) each day over a number of days. However, G-CSF-induced mPB does not always yield enough cells for a graft, takes 4–5 days for optimal yield of HSC and HPC, and can entail leukocytapheresis efforts to obtain enough cells for a transplant. This requires the donor to be ready and willing for multiple days of G-CSF treatment. Moreover, there are patients who do not mobilize HSC and HPC well for a number of reasons, including low numbers of these cells inherent in the BM of the patient undergoing an autologous transplant—an example being patients with Fanconi anemia, under such conditions, one might attempt to use gene therapy on the mobilized cells to correct the Fanconi anemia gene defect. New, simple, and less time-consuming efforts to mobilize HSC and HPC for clinical use would be advantageous. In this context, a recent article by Hoggatt et al.[6] describes a new regimen combining two small molecules (GRO-beta and AMD3100) to quickly mobilize HSC and HPC in mice.

As a background to the article by Hoggatt et al.,[6] a number of small molecules have been used in mice and/or man, alone or in combination with G-CSF, to enhance collection of HSC and HPC with an optimized mobilization procedure. This includes the chemokine, macrophage inflammatory protein (MIP)-1 alpha (also referred to as CCL3),[7–10] which, although active as a mobilizer in mice, did not show much, if any, efficacy in humans when its analog BB10010 was used.[7–10] Among a number of small molecules that have been used to mobilize HSC and HPC, one that stands out and has been used for good advantage is AMD3100 (Plerixafor).[3–16] AMD3100 has been used alone but synergizes with G-CSF to greatly enhance mobilization of HSC and HPC in both mice and humans.[11–14] The combination of the two usually entails 4 days or more of G-CSF given twice a day with AMD3100 given on the last day of G-CSF administration, and the cells are collected hours later. Another small molecule, the chemokine growth-regulated protein (GRO)-beta (also referred to as CXCL2), has shown some efficacy in mice and rhesus monkeys[17] and now in humans[6] as a mobilizer of HSC and HPC, although the GRO-beta by itself in humans had only modest effects.[6] In contrast to G-CSF, which takes days to mobilize sufficient HSC and HPC, AMD3100 and GRO-beta, each when given alone, act quickly in mice within 15 minutes to a few hours.

In an effort to more quickly and simply mobilize HSC and HPC, groups from the Indiana University and the Harvard University collaborated to assess the combined mobilizing effects of GRO-beta and AMD3100 in mice.[6] Take-home messages from their article were several fold: A single injection of both GRO-beta, which acts as an agonist through the C-X-C chemokine receptor type 2 (CXCR2) on neutrophils resulting in release of metalloproteinase (MMP) 9,[6] and AMD3100, which can act as an antagonist of the stromal cell-derived factor (SDF) 1/C-X-C chemokine ligand 12 (CXCL12)-CXCR4 receptor axis interaction[11–14] but which also manifests agonistic effects on this latter interaction,[18] results in mobilizing the same numbers of HSC and HPC in mice within 15 minutes, which takes days to mobilize using G-CSF. Of significance and high biological interest, they noted that the combination of GRO-beta and AMD3100 mobilized a higher engrafting and competitive HSC population than that of G-CSF.[6] This may not be too surprising a find, because there was some evidence that AMD3100 alone and in combination with G-CSF also seems to mobilize a high engrafting HSC population.[11]

As exciting as their mouse data[6] is, the combination of MIP-1-alpha plus AMD3100 plus G-CSF was a more potent mobilizer in mice than either combination of two alone,[9] and whether or not such an efficacious mobilization procedure using the combination of GRObeta and AMD3100 will work in humans remains to be seen. It may take a while to sort out the best mobilization timing in humans. While GRO-beta seems to work within 15 minutes in mice and humans as a mobilizer,[6] AMD3100 takes a much longer time to mobilize in humans than in mice (e.g., AMD3100 in mice peaks at about 1 hour but takes 9–12 hours in humans for maximal effectiveness).[11–14] However, sorting this window of timing out for the combined use of GRO-beta and AMD3100 in humans should not be too difficult.

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A most intriguing aspect of the work by Hoggatt et al.[6] is the characteristics of the cells mobilized by the combination of GRO-beta plus AMD3100. What exactly is this higher engrafting and competing HSC population? RNA sequencing found that these highly engrafting and competitive mouse cells have a distinct transcriptome different from that of G-CSF mobilized mouse cells,[6] but it is not yet clear if these differences can be defined with regard to the surface phenotype of HSC to separate out these highly engrafting from lesser engrafting HSC. Cluster density (CD)166 is a type 1 cell surface transmembrane glycoprotein that is a member of the immunoglobulin superfamily of molecules.[19,20] CD166 is present on the surface of mouse and human HSC as well as on the regulatory cells in the microenvironment niche. Perhaps CD166 may be a usable surface marker candidate when added to the most rigorously defined phenotypic HSC populations to distinguish differentially engrafting cells. Can adding CD166 as a marker further define this highly functional HSC population in mice and man, and what about other cell surface functional markers such as CXCR4, the homing and survival receptor for SDF-1/CXCL12,[21–26] and dipeptidyl peptidase (DPP) 4 that can truncate and change the activities of a number of biologically active proteins including SDF-1/CXCL12?[27–31] How well these additional markers work for human cells to define the more efficacious engrafting cells remains to be determined and may well depend on the expression levels of these surface proteins.

Once the characteristics of these highly engrafting cells are known, the question becomes if this engrafting ability can be further enhanced or are these cells already maximally fit for engraftment? A number of different measures have been used to enhance engraftment of CB HSC, in part through enhancing the expression of CXCR4 on the cells and increasing the homing capacity of these cells. This includes using short-term ex vivo pulsing periods (e.g., hours) with inhibitors of DPP4[27,30] or of the enzyme histone deacetylase (HDAC)5[32] and/or use of prosta-glandin E (PGE),[33,34] glucocorticosteroids (e.g., dexamethosone or Flonase),[35] or hyperthermia treatment[36] of the donor cells, as well as short-term DPP4 inhibitor administration to the recipient before administration of the donor cells.[30,37–39] Combined PGE pulsing of mouse BM donor cells into DPP4-inhibitor-treated recipients has already been shown to enhance engraftment of mouse BM cells into lethally irradiated mice.[40]

Efforts to enhance the collection and engrafting capability of donor cells are ongoing in numerous laboratories worldwide. We have found that collecting mouse BM and human CB cells under hypoxic conditions (e.g., at 3% oxygen) or in ambient air in the presence of cyclosporine A mitigates the effects of extra physiological oxygen shock/stress (EPHOSS) that acts through a cyclophilin D–P53–mitochondrial permeability transition pore axis, which involves the release of reactive oxygen species, hypoxia-inducing factor, and the hypoximer, miR210.[41] EPHOSS, upon collection of cells in ambient air, causes rapid loss of HSC through a differentiation, rather than cell death, phenomenon. Would such collections of the mPB in hypoxia or in air in the presence of cyclosporine A or combinations of inhibitors of antioxidants and/or epigenetic enzymes[42] enhance numbers of GRO-beta and AMD3100 mPB HSC?

It is only through rigorous experimentation that we will enhance the efficiency of HCT using not only mPB but also BM and CB. However, a note of caution is necessary when trying to move from mouse studies to human trials. Some efforts that work in humans do not seem to work in mice.[32,35] For example,

glucocorticosteroids and HDAC5 inhibitors worked with human, but not mouse, cells and it is possible that certain efforts that work in mice will not work with humans, or the timing differences for optimal efficacy may vary. The only way to know for sure is to do the experiments and then test them to determine if they work in humans.

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