IN VITRO MEGAKARYOCYTOPOIESIS

To the Editor:

Choudhury et al[1] recently described a liquid culture system derived from adult hamster spleen for the study of megakaryocytopoiesis. These cultures can be maintained for more than four months.[2] I think it is important to note that long-term cultures of hamster bone marrow will also maintain megakaryocytopoiesis at similar or even higher levels in the absence of an adherent stromal layer for as long as four months.[3,4] The authors stated that "the importance of reasonably consistent levels of megakaryocytes in the supernatants of these cultures lies in the fact that differentiated megakaryocytes have not been demonstrated for extensive periods in other in vitro systems." This is definitely not the case. In long-term hamster bone marrow cultures as many as six percent of the cells are acetylcholinesterase positive. The advantage of the bone marrow system is that under the right conditions, cells can be grown in suspension in the absence of the adherent layer.[3,4] Whichever system is used, it appears that both hamster spleen and bone marrow provide useful models for the in vitro study of megakaryocytopoiesis.

CHRISTINE EASTMENT
Transplantation Laboratory
Old Georgetown Road Facility
9312 Old Georgetown Rd
Bethesda, MD 20814

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To the Editor:

Dr Eastment's letter is acknowledged. During a previous study[1] and during preliminary work for this study,[2] we were unable to maintain megakaryocytopoiesis consistently in the primary suspension cultures of hamster bone marrow, for the same periods as in primary cultures of hamster spleen cells. Both varieties of primary cultures were grown under similar conditions, as described in our report.[2] Therefore, in our hands, primary cultures of hamster spleen provided the more consistent method for investigation of megakaryocytopoiesis.

CHANDRA CHOUDHURY
Associate Professor
Division of Hematology/Oncology
Southern Illinois University
School of Medicine
PO Box 3926
Springfield, IL 62708

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LECTIN AGGLUTINABILITY OF THE HUMAN HEMATOPOIETIC STEM CELL

To the Editor:

In their otherwise excellent studies, Ebell et al[1] indicate that hemopoietic stem cells (HSC) are not agglutinable with the lectin soybean agglutinin, implying that these cells do not have surface glycoproteins terminating in galactosyl or *N*-acetyl galactosaminyl residues. This notion is erroneous and potentially misleading.

In the murine system, there is ample evidence that HSC are agglutinable with both soybean agglutinin (SBA) and peanut agglutinin (PNA), indicating the presence of surface glycoproteins terminating in galactosyl residues. In their elegant study, Reisner et al[2] demonstrated that the CFU-S potential of marrow and spleen cells was significantly reduced after agglutination with SBA and PNA. Using fluorescence microscopy, Nicola et al[3] have also demonstrated the presence of SBA and PNA receptors (ie, galactosyl residues) on murine CFU-Mix as well as the CFU-C. They could further agglutinate the latter cell with SBA. We have also confirmed the agglutinability of murine CFU-C by SBA. In the human system, an assay for the HSC, equivalent to CFU-S, does not exist. The in vitro CFU-Mix may be analogous but studies have not been done to find out if human CFU-Mix is agglutinable by SBA and PNA. Reisner et al[4] have shown that human CFU-C is not agglutinable by SBA, and in this regard the human system may be different from the murine system. But this finding does not reflect on the agglutinability of the HSC.

The importance of this question lies in its application to bone marrow transplantation where SBA can be used to deplete the marrow of T lymphocytes and thereby reduce the incidence and severity of graft-*v*-host disease (GVHD). If HSC are also agglutinated during this procedure, the procedure can negate the objective of transplantation. In fact, the failure or delayed hemopoietic reconstitution observed in some recipients of lectin-treated marrow grafts[5] could indeed be attributed to the depletion of HSC by this treatment rather than the depletion of stromal cells as Ebell et al seem to imply.

The question has another ramification. Transplanted HSC must specifically lodge in the hemopoietic compartment of the recipient's marrow.[6] Since the hemopoietic compartment is extravascular, the lodging requires transendothelial passage of HSC which is the result

of membrane interaction between HSC and marrow endothelium. The specificity of this interaction is thought to be determined by membrane glycoproteins and lectin-like substances. Thus, attention has been recently focused on mapping of marrow endothelium and HSC for glycoproteins and lectin-like substances. Lectin mapping of marrow endothelium has been reported by our own group[7] as well as by Pino.[8] Furthermore, we have recently identified a lectin-like substance on the plasma membrane of marrow sinus endothelium capable of recognizing galactosyl residues.[9] It is entirely possible that the selective lodging of HSC in the marrow is mediated by interaction between this lectin-like substance and galactosyl-terminating glycoproteins on the surface of HSC.

MEHDI TAVASSOLI
SHIN AIZAWA
Veterans Administration Hospital
University of Mississippi Medical Center
Jackson, MS 39216

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To the Editor:

We appreciate the comments of Drs Tavassoli and Aizawa on our article.[1] In this article, however, we did not state that a general property of hematopoietic stem cells is their lack of cell surface glycoproteins terminating in galactosyl or *N*-acetyl galactosaminyl residues. Rather, we state that human hematopoietic progenitors, unlike murine progenitors, differ from mature cells in their expression of these residues. As they point out, there is ample evidence in the murine system that CFU-S, CFU-Mix and CFU-GM are agglutinable with soybean agglutinin (SBA).[2,3] In contrast, human hematopoietic progenitors do not possess these residues on their cell surface in sufficient density to be agglutinated by this lectin, whereas mature cells (eg, T lymphocytes, neutrophils, etc) do. Thus, human CFU-GM are not agglutinated by SBA.[1,4] Although it is correct that this property may not reflect those of early stem cells, it clearly shows that human cells do not behave like mouse cells. Human CFU-Mix are also not agglutinated by SBA (Lu L, et al, manuscript in preparation). Again, we recognize that CFU-Mix may not be the cells that engender hematopoietic reconstitution. For example, we and others[5,6] have recently shown that marrow grafts depleted of CFU-Mix by treatment with 4-hydroperoxycyclophosphamide can still reinstitute full hematopoietic function in supralethally irradiated recipients. These clinical observations suggest that there are early pluripotent stem cells different from CFU-Mix that are not affected by this treatment and are responsible for hematopoietic reconstitution of the transplanted host.

The most convincing evidence that the earliest human hematopoietic stem cells responsible for reconstitution of marrow function are SBA-negative comes from the observations on bone marrow transplantation using SBA-treated marrow grafts. Contrary to Drs Tavassoli and Aizawa's impression, hematopoietic reconstitution in the patient cited in our original report[7] was rapid and not different from that observed following a conventional marrow graft. Similarly, in recipients of HLA-identical grafts depleted of T cells and stromal elements by the method of Reisner, we have observed a complete and sustained reconstitution of hematopoietic function in 20 of 22 cases following preparation with 1,320 rad of total body irradiation and 120 mg/kg of cyclophosphamide.[8] That stem cells are indeed transplanted is suggested from our initial report,[7] in which the patient, who relapsed with her own leukemia, was able to recover normal donor type hematopoiesis following two separate courses of intensive chemotherapy used to treat this relapse.

In our first series, only two patients rejected HLA-matched grafts.[8] In HLA mismatched marrow grafts for leukemia, a higher incidence of graft failure or rejection was observed.[8] We considered stromal cell depletion from the graft as a possible cause for failure of engraftment in certain cases. This mechanism may contribute to graft failure if the patient, by virtue of prior therapy or other mechanisms, has an inadequate microenvironment to support hematopoietic engraftment. We indeed have evidence for such a mechanism in one case (Castro-Malaspina H, et al, manuscript in preparation). In almost all cases, however, graft rejection results from an active resistance mediated by radioresistant host lymphocytes. We and others[9,10,11] have shown that there are indeed host radioresistant lymphocytes exhibiting cytotoxicity against donor specificities. Abrogation of resistance by increasing pretransplant immunosuppression has overcome the active cell mediated mechanism responsible for rejection or delayed hematopoietic reconstitution.[12] Taken together, results from our bone marrow transplantation experience in humans clearly demonstrate that primitive hematopoietic stem cells reside in the cell fraction not agglutinated by SBA. Thus, we conclude that galactosylterminating glycoproteins are absent or in low density on the cell surface of human hematopoietic progenitors.

We agree with Drs Tavassoli and Aizawa that membrane glycoproteins may play a significant role in the process of recognition and lodgement of early hematopoietic stem cells in the marrow tissue.

HUGO CASTRO-MALASPINA
RICHARD J. O'REILLY
Bone Marrow Transplantation Service
Memorial Sloan-Kettering Cancer Center
New York, NY 10021