

This Week's Citation Classic

Pike B L & Robinson W A. Human bone marrow colony growth in agar-gel. *J. Cell. Physiol.* 76:77-84, 1970.
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This article describes a method for the growth of colonies from human bone marrow aspirates *in vitro* in agar-gel medium. 'Feeder layers' containing normal human peripheral blood leukocytes were used to stimulate colony development. Colonies generated from normal human bone marrow cells were almost exclusively granulocytic. [The SCⁱ® indicates that this paper has been cited in over 835 publications since 1970.]

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April 27, 1983

"Both Bill Robinson and I learned the basics of *in vitro* culture of bone marrow cells at the clonal level from Donald Metcalf and Ray Bradley during time spent at the Walter and Eliza Hall Institute in Melbourne. Bill returned to Denver and I with him, and we took up the challenge of developing a simple culture system for colony development from single human bone marrow cells. Use of 'feeder layers' of a variety of cell types such as kidney tubules, as described by Bradley and Metcalf,¹ to support colony growth from murine bone marrow cells failed to stimulate colony growth from human cells. Sources of colony-stimulating activity (CSA), such as human urine, capable of stimulating colony development from murine bone marrow cells² were also totally inactive on human cells.

"At this time, we were documenting urinary CSA levels in leukemic patients to see if any correlation existed between CSA levels and the clinical course of the disease. It became readily apparent that a rough inverse correlation between the proportion of blast cells in the peripheral blood and CSA

levels existed. Untreated patients with acute granulocytic leukemia exhibited low levels on presentation, which rose during remission following chemotherapy.³ The knowledge that CSA activity rose concomitantly with the mature peripheral blood granulocyte levels led us to draw up a working model for the regulation of granulopoiesis by-products from mature granulocytes.

"To test our hypothesis, we titrated normal human peripheral blood buffy coat leukocytes into an agar-underlayer and added murine bone marrow cells in an overlayer in the presence and absence of a known source of CSA. As a human bone marrow aspirate sample was available at the time, it was set up simultaneously. We had tried so many variants and the best we hoped for was inhibition of colony growth of murine bone marrow with high numbers of peripheral white blood cells. I can well remember our excitement when a few days later we noted colony growth in the absence of added CSA. Furthermore, it appeared that the human marrow cells were also beginning to proliferate, and after a few more days, discrete colonies were visible.

"It was with great excitement, and some degree of courage, that we repeated the experiment. Our initial observations were confirmed. Human peripheral blood leukocytes provided adequate stimulation to allow single human bone marrow cells to proliferate to form granulocytic colonies. The solution to the problem was in fact quite simple.

"The reason this paper has been cited so frequently is that it provided the first simple, reproducible method for the generation of colonies from single human bone marrow cells *in vitro* (and subsequently also from human peripheral blood cells). This allowed studies of the regulation of granulopoiesis in both the normal and leukemic situations. The technique has been used clinically to classify leukemias by using the growth characteristics of cells as a criterion, to assess possible responses to chemotherapy, and also to assess remission status. Its overall contribution to the understanding of the growth characteristics of both normal and leukemic cells has been extensively covered in a publication by Metcalf."⁴

1. Bradley T R & Metcalf D. The growth of mouse bone marrow cells *in vitro*. *Aust. J. Exp. Biol. Med. Sci.* 44:287-300, 1966.
[Citation Classic. *Current Contents/Life Sciences* 22(40): 12, 1 October 1979.]
2. Robinson W A, Stanley E R & Metcalf D. Stimulation of bone marrow colony growth in vitro by human urine. *Blood* 33:396-9, 1969.
3. Robinson W A & Pike B L. Leukopoietic activity in human urine: the granulocytic leukemias. *N. Engl. J. Med.* 282:1291-7, 1970.
4. Metcalf D. Hemopoietic colonies: in vitro cloning of normal and leukemic cells. *Rec. Res. Cancer Res.* 61:1-227, 1977.