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This Week's Citation Classic

Iscove N N, Senn J S, Till J E & McCulloch E A. Colony formation by normal and leukemic human marrow cells in culture: effect of conditioned medium from human leukocytes. *Blood* **37**:1-5, 1971. [Inst. Med. Sci., Univ. Toronto; Ontario Cancer Inst.; and Sunnybrook Hosp., Toronto, Canada]

'Conditioned medium' obtained from cultures of human peripheral leukocytes promoted the growth of human marrow cells in cell culture. This material also permitted the growth of small colonies fromthe marrow of patients with acute myelogenous leukemia in relapse; in its absence, only occasional colonies were observed. [The *SCI*® indicates that this paper has been cited in over 390 publications since 1971.]

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"Research into the precursors of blood cell formation was transformed in the 1960s by two developments. First, there was the introduction of the spleen colony assay for primitive hemopoietic precursors in the mouse by Till and McCulloch in 1961,[1] demonstrating the power of clonal analysis applied to precursor cells too rare to be examined directly. This development was soon followed, in the mid-1960s, by the successful adaptation of tissue culture methods to the clonal detection of granulocyte and macrophage precursors in the mouse by Pluznik and Sachs[2] and by Bradley and Metcalf.[3]

"By the time I began my graduate studies with McCulloch in 1968, many investigators were anxious to have culture techniques which would work with human material. In the previous year, Senn reported with McCulloch and Till the first success in culturing human granulocyte precursors from bone marrow.[4] The colonies were quite small, but within a short time Pike and Robinson had achieved significantly better growth conditions in agar over a 'feeder layer' of human blood leukocytes.[5] Tissue culture methodology was in rapid flux at the time. The requirement of mouse granulocyte and macrophage precursors for glyco-protein growth factors ('colony-stimulating activity,' CSA, or 'colony-stimulating factor,' CSF) was becoming apparent, and feeder layers producing CSA were in the course of being replaced by medium 'conditioned' by appropriate cellular sources of these activities. It seemed likely that human cells would have the same requirement. In my host laboratory, methyl cellulose had been found to have certain technical advantages over agar, including a greater sensitivity of detection of CSA of mouse origin. I therefore set out, with Senn, McCulloch, and Till, to adapt the methyl cellulose method to the culture of human marrow cells and to replace the feeder layers with an appropriate conditioned medium.

"In our first attempts, colonies did not grow at all in the same conditions which worked so well with mouse cells. In particular, conditioned medium derived from mouse cells and active on mouse cells had no effect on human marrow cells. Finally, two elements, both inspired by the Pike and Robinson method, proved effective. The first was the inclusion of asparagine in our culture medium, a 'nonessential' amino acid thought at the time to be a requirement for leukemic but not normal cells. It proved essential for colony growth by normal human hemopoietic precursors. The second was the use of conditioned medium from human blood leukocytes as the source of CSA. This step was not straightforward, since medium from normal leukocytes incubated alone in liquid culture had only weak or undetectable activity. However, medium cultured over a feeder layer of leukocytes immobilized in agar did work. Such 'conditioned medium' was reported in our 1971 paper and became a standard constituent of our cultures. Later, phytohaemagglutinin was found to stimulate leukocytes to release activity in liquid culture, and more recently the original observations were explained by the finding by Hoang *et al.* that a stimulus to activity release can be extracted from the crude agar itself.[6]

"The high frequency of citation of this paper along with the one by Pike and Robinson[5] and similar studies in the same period by Chervenick and Boggs[7] and by Paran and Sachs et a/.[8] reflects the fact that these studies initiated the era of application of clonal tissue culture to human hematopoietic precursors, demonstrated the existence of granulopoietic CSFs for both normal and leukemic human cells, and showed that leukemic cells could grow in culture and would therefore be amenable to direct study."

1. **Till J E & McCulloch E A.** A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.* **14:**213-22, 1961. (Citation Classic. *Current Contents/Life Sciences* **22**(43):12, 22 October 1979.]
2. **Pluznik D H & Sachs L.** The cloning of normal "mast" cells in tissue culture. J. *Cell. Comp. Physiol.* **66**:319-24, 1965.
3. **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.]
4. **Senn J S, McCulloch E A & Till J E.** Comparison of colony-forming ability of normal and leukaemic human marrow in cell culture. *Lancet* **2**:597-8, 1967.
5. **Pike B L & Robinson W A.** Human bone marrow colony growth in agar-gel. *J. Cell. Physiol.* **76**:77-84, 1970. [Citation Classic. *Current Contents/Life Sciences* **26**(23): 19, 6 June 1983.]
6. **Hoang T, Iscove N N & Odartchenko N.** Agar extract induces release of granulocyte colony-stimulating activity from human peripheral leukocytes. *Exp. Hematol.* **9**:499-504, 1981.
7. **Chervenick P A & Boggs D R.** Bone marrow colonies: stimulation *in vitro* by supernatant from incubated human blood cells. *Science* **169**:691-2, 1970. (The *SCI* indicates that this paper has been cited in over 120 publications since 1970.)
8. **Paran M, Sachs L, Barak Y & Resnitzky P.** *In vitro* induction of granulocyte differentiation in hemopoietic cells from leukemic and non-leukemic patients. *Proc. Nat. Acad. Sci. US* **67**:1542-9. 1970. [The *SCI* indicates that this paper has been cited in over 255 publications since 1970.]