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This Week's Citation Classic

Pluznik D H & Sachs L. The cloning of normal "mast" cells in tissue culture.
J. Cell. Comp. Physiol. **66**:319-24, 1965.
[Section of Genetics, Weizmann Institute of Science, Rehovot, Israel]

The findings published in this paper show that it is possible to obtain clones and clonal differentiation of normal hematopoietic cells in soft agar. The formation of these clones and their differentiation required specific inducing substance(s) produced by other cells that were seeded underneath the agar. It is concluded that a similar approach should be applicable to the *in vitro* cloning and clonal differentiation of all the different types of hematopoietic cells. [The *SCI*® indicates that this paper has been cited in over 590 publications since 1965.]

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"My research with hematopoietic cells began with the aim to develop cell culture systems for the cloning and clonal differentiation of specific cell types, as an approach to studying the controls that regulate growth and differentiation of normal and malignant cells. My first paper on hematopoietic cells *in vitro*, in 1961, with a graduate student, H. Ginsburg, described the culture of leukemic cells using spleen feeder layers.[1] I also carried out experiments with normal cells and the next paper, in 1963, with the same student, described the clonal growth and differentiation of normal mast cells and granulocytes in liquid medium using fibroblast feeder layers to provide the necessary inducers for this clonal growth and differentiation.[2] I wrote as the concluding sentence in this paper: 'The described cultures thus seem to offer a useful system for a quantitative kinetic approach to hematopoietic cell formation and for experimental studies on the mechanism and regulation of hematopoietic cell differentiation.'[2]

"This 1963 paper led to such an approach. It was then shown in the 1965 paper with another graduate student, D.H. Pluznik (now at the National Institutes of Health), that normal hematopoietic cells can be cloned in soft agar and that the formation of these clones required an inducer(s) secreted by the feeder layer cells which had been seeded underneath the agar. Since the cells in agar showed some morphological resemblance to the mast cells which had previously been studied, they were provisionally labeled as 'mast cells' in this paper. However, our other results showed that they were macrophages.

"These two papers in 1963 and 1965 thus described the first *in vitro* system for cloning and clonal differentiation of specific types of normal hematopoietic cells, and showed that the induction of such clones was dependent upon inducers secreted by other cells. Our agar assay for cloning normal hematopoietic cells was confirmed by Bradley and Metcalf in 1966.[3] In the next step it was found that the inducers required for the production of macrophage and granulocyte clones were present in conditioned medium produced by the feeder cells, that there appeared to be a different inducer for macrophage and granulocyte clones, and that these cells could also be cloned in methylcellulose.[4] Cloning in agar and methylcellulose are the two main procedures which are now used. The use of appropriate human conditioned medium extended the cloning assay to human hematopoietic cells, showed that the appropriate normal inducer could induce differentiation of some human myeloid leukemic cells, and suggested that induction of normal differentiation of leukemic cells could be a useful approach to therapy.[5]

"This *in vitro* approach has led to the cloning and isolation of growth factors for all types of hematopoietic cells, including different types of lymphocytes. It also led to a further understanding of the controls that regulate growth and differentiation in hematopoiesis, how these controls are coupled in normal development, and the abnormalities in these controls in leukemia and other hematological abnormalities.[6] This can explain why this 1965 paper has been highly cited."

1. **Ginsburg H & Sachs L.** The long-term cultivation in tissue culture of leukemic cells from mouse leukemia induced by Moloney virus or by x-rays. *J. Nat. Cancer Inst.* **27**:1153-71, 1961.
2. **----------.** Formation of pure suspensions of mast cells in tissue culture by differentiation of lymphoid cells from the mouse thymus. *J. Nat. Cancer Inst.* **31**:1-40, 1963.
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. **Ichikawa Y, Pluznik D H & Sachs L.** *In vitro* control of the development of macrophage and granulocyte colonies. *Proc. Nat. Acad. Sci. US* **56**:488-95, 1966.
[The *SCI* indicates that this paper has been cited in over 260 publications since 1966.]
5. **Paran M, Sachs L, Barak Y & Resnitzky P.** *In vitro* induction of granulocyte differentiation in hematopoietic 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 250 publications since 1970.]
6. **Sachs L.** Normal developmental programmes in myeloid leukemia. Regulatory proteins in the control of growth and differentiation. *Cancer Surveys* **1**:321-42, 1982.