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This Week's Citation Classic®

Allen T D & Dexter T M. Cellular interrelationships during in vitro granulopoiesis. *Differentiation* 6:191-4, 1976.
[Paterson Laboratories, Christie Hospital and Holt Radium Institute, Withington, Manchester, England]

This paper contained the first ultrastructural observations of cell types and their interactions in mouse long-term bone-marrow cultures. In this system, nonhaematopoietic stromal cells proliferate and provide the microenvironmental conditions necessary for the continued proliferation and differentiation of haematopoietic stem cells over periods of several months. [The *SCI*® indicates that this paper has been cited in over 160 publications, making it the most-cited paper for this journal.]

T.D. Allen
Department of Ultrastructure
Paterson Institute for Cancer Research
Christie Hospital & Holt Radium Institute
Manchester M20 9BX
England

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This work was an early set of observations on an *in vitro* system for maintaining bone marrow that came to be known as "Dexter Culture" and has subsequently become established worldwide as a basic technique in experimental haematology. At the time, Mike Dexter was working in the Experimental Haematology group led by Laslo Lajtha, the director of the Paterson Labs, and I was in charge of the Electron Microscope Unit. At this stage the marrow culture system had not yet reached its final sophistication for optimal growth,[1] and an ultrastructural survey of the cell types present was thought to be a worthwhile exercise.

A simple marker of haematopoietically active cultures was the presence of giant adipocytes in the adherent layer, which grew in foci large enough to be visible to the naked eye, and we felt it was important to know if there was any direct involvement of these cells in the haematopoietic production. Also, although the idea of specific microenvironments had been suggested previously for bone marrow and spleen *in vivo*,[2] the general view in haematology for stem cell commitment and differentiation was as a stochastic process, subject to hormonal (subsequently growth factor) influences, but not necessarily involving the stromal cells *per se*. Perhaps one of the reasons for the frequent citation of this article has been the general realisation of stromal cell involvement in haematopoietic processes.

Technically, the investigation posed interesting problems for *in situ* electron microscopy of the cell types, as at the time the cultures were maintained in glass medical flasks. Separation of the embedding resin from the glass involved a firm blow with a hammer and alternate dipping of the fragments in boiling water and liquid nitrogen, which was suitable for sectioning. Scanning microscopy preparation was simpler but similarly crude, as speed was necessary to prevent air drying.

It is encouraging that the cell types and cellular interactions that we characterised in morphological terms at the time have been shown to be substantially correct in the light of a subsequent barrage of histochemical and immunocytochemical investigations. At one time we were alone in our belief that the lipid-laden cells were truly adipocytes and not lipid-engorged macrophages, a point that Paul Simmons, a graduate student in the labs, finally confirmed some eight years later, using immunoelectron microscopy.[3]

The long-term marrow culture continues to be the focus of collaboration between the Department of Experimental Haematology, now headed by Dexter, and my own Department of Ultrastructure. We are currently investigating the dynamic aspects of the cellular interactions using time-lapse video techniques. These studies have already indicated that the biological "mass production" of blood cells (4×10^{11} per day in humans) can in some ways mirror mass production in general, with production lines, quality control, and delivery systems.

The marrow culture system has also recently made a significant contribution to cancer treatment under the guidance of Dexter and clinical colleagues. For some as yet undefined reason, when cultures are established using marrow cells from some patients with acute myeloblastic leukaemia, the leukaemic cells are lost while the normal cells grow. Reinfusion of these leukaemia-free cells into the patient following ablative therapy (autologous bone-marrow transplantation) is showing great promise in the treatment of this disease.[4]

As a final note, this article was rejected for publication by *Nature*, a fate perhaps shared with more than one *Citation Classic*.

1. Dexter T M, Allen T D & Lajtha L G. Conditions controlling the proliferation of hematopoietic stem cells in vitro. *J. Cell Physiol.* 91:335-44, 1977. (Cited 550 times.)
2. Trentin J J. Influence of hematopoietic organ stroma (hematopoietic microenvironment) on stem cell differentiation. (Gordon A S, ed.) *Regulation of hematopoiesis*. New York: Meredith, 1970. Vol. 1. p. 161-86. (Cited 145 times.)
3. Dexter T M, Spooncer E, Simmons P J & Allen T D. Long-term marrow culture: an overview of techniques and experience. (Wright D G & Greenberger J S, eds.) *Long-term bone marrow culture: proceedings of a symposium*. New York: Liss, 1984. p. 57-96.
4. Chang J, Morgenstern G, Deakin D, Testa N G, Coutinho L, Scarffe J H, Harrison C & Dexter T M. Reconstitution of haemopoietic system with autologous marrow taken during relapse of acute myeloblastic leukaemia and grown in long-term culture. *Lancet* 1:294-5, 1986.