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50 Years Later: Remembering the Paper

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Sometimes a moment in your life is so important you remember it in startling detail—where you were, who was there, what happened. As a first-year medical student in my first months of research at Stanford, I was walking down the hall of the basement radiation biology labs at Stanford when Henry Kaplan, my mentor and benefactor, stepped out of his lab and motioned me over. In his right hand, for some of you memorable in itself, he held the new issue of *Radiation Research*. He pointed to the now famous paper (*1*) and said "You should read this; it will be important." I spotted the bumps on the spleen and wondered if I would have been astute enough to follow it up rather than trashing the finding as some artifact or infection. Later, when I read the paper, I was struck at how precise the quantitative data on numbers of cells to make a day 10 spleen colony was from an *in vivo* assay and how precise the radiation sensitivity tests could be. Real *in vivo* biology could be quantitative. But the major finding came in just a few sentences—each colony had at least four blood cell types (monocytic, granulocytic, erythroid and megakaryocytic), and they proposed that each colony was derived from a single clonal progenitor. They mentioned that the spleen colony-forming cell was probably from an undifferentiated cell.

The term stem cells was not mentioned, but then we had no important definition of such a cell at that time. But the idea of a multilineage clonal progenitor was exciting, and little did I know then how their elegant subsequent papers would establish the concept and set most of the rules. The demonstration that day 10 CFU-s cells were clonal came from one of the most innovative experiments I have read in my lifetime in science—irradiate the donor cells and check for unique chromosomal aberrations in the survivors, and if all dividing cells in a colony had the same random and unique marker, which they did, the colonies were clonal (*2*). Then the demonstration that sometimes day 10 colonies had also produced many day 10 CFU-s clonal progenitors led to the idea of at least short-term self-renewal (*3*). Finally, the use of this technique to follow cells a little longer and to show lymphocytes could be part of the clone revealed that the bone marrow contained infrequent cells capable of multilineage myeloerythroid and sometimes lymphoid maturation, and at least some of these could self-renew for the time intervals studied (*4*). By then the term stem cells was being used, in fact pluripotent hematopoietic stem cells (a term changed to recognize the potency of ES cells). I knew from then that any cell isolated that could do less than self-renew as well as give rise in its clonal progeny to all known blood cells types would not make the grade as a stem cell.

When later I came back to the identification and prospective isolation of HSC following the lead of pursuing T- and B-lymphocyte progenitors (*5–9*), we had established clonal assays for all progenitors. We started with a thymic colony assay that also measured clonogenic marrow cells (*10–12*), a clonal assay for B-lymphocyte progenitors (*13*) on clonal stromal cells taken from Whitlock-Witte cultures (*14*), and myeloerythroid colony-forming cells at day 12–14 in the spleen; we followed the Iscove *et al.* correction of the time of separate progenitors to form day 8 or 10 or 12–14 splenic colonies (*15*). [We later showed that oligolineage progenitors gave rise to day 8 CFU-s, and mainly multipotent progenitors and HSC to day 12 CFU-s (*16*)]. And, like others, we found that long-term multilineage engraftment of all blood cell types in lethally irradiated hosts was another assay (*13*), as was retransplantation from purified cells of a particular phenotype. With the availability of the hybridoma techniques to get a constant reagent for a constant cell surface epitope (*17*), and the fluorescence-activated cell sorter (*18*), we could isolate marked marrow cells for simultaneous assay in all of the tests described above. We reported high enrichment of HSC (*13*), then even higher (*19, 20*), and over the next 20 years many HSC subsets and nearly all of the downstream progenitors in mice (*21–25*) and in humans (*26–28*). Much to our delight, both mouse and human HSC markers were so unique we could isolate T-cell-free HSC for allogeneic transplantation without GvH (*29–30*) and cancer-free HSC from patients with widespread breast cancers or lymphomas, so that HSC rescue after myeloablative chemotherapy could be done without re-introducing cancer cells to the patients [(*31, 32*), Mueller *et al.*, Long-term followup of patients with metastatic breast cancer treated with high-dose chemotherapy and transplantation of highly purified hematopoietic stem cells, manuscript in preparation]. So it

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was clear to me that the field initiated by Till and McCulloch in the 1960s could be the basis for many clinical therapies, including regenerative medicine for a number of tissues when the stem cell assays and isolation method was extended to these other tissues (*33–35*).

That is the field established by a biophysicist and hematologist-oncologist wishing to develop an assay of normal tissue radiosensitivity to compare with cancer radiosensitivity by injecting bone marrow cells into mice and seeing bumps. They were wise enough to recognize far more than pathology to explain the bumps and had the vision and the experimental innovation to show that HSC exist, and they and their school of stem cell biology and medicine in Toronto have provided the world with the most remarkable field.

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