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Clonal Origins of the Hematopoietic System: The Single Most Elegant Experiment

Irving L. Weissman

The paper by Becker et al. (1) is the single most beautiful paper I have read. It follows what, in my view, is the most important single paper to the communities of immunology, hematopoiesis, and stem cell biology by Ray Owen in 1945 (2). This paper established that hematopoietic progenitors, or stem cells, must exist and must enter the circulation available to fraternal calf twins that share a single placenta, and that the circulated and engrafted blood-forming cells induced tolerance to themselves if they engrafted in utero. In contrast, postnatal transplantation of blood cells led to immunity, not tolerance.

If blood-forming precursors could naturally engraft in these freemartin cattle that shared a placenta, surely other means could reveal the transplantability of blood formation and the types of cells that might be involved. Following the atomic bomb explosions among civilian populations in Hiroshima and Nagasaki, also in 1945, it became clear in animal experiments to reproduce whole body radiation that the lowest lethal dose effectively eliminates the blood-forming system, resulting in death ~2 wk after the radiation. Blocking radiation to all the bones or spleen with lead shielding could prevent hematopoietic death. This could also be accomplished by bone marrow transplants to the recently irradiated host mice. The dominant explanation at the time was that factors released by the unirradiated bone/spleen or transferred marrow cells caused repair of radiation lesions in host cells (3). This notion was reversed by two important papers using as marrow donors either mice bearing a chromosomal marker or rats with their own trackable chromosomes and enzymes, showing that at least early post-treatment survival was driven by the regeneration of blood formation by donor cells rather than by host cells repaired by the postulated factors (4, 5).

In 1961 a radiation physicist, James Till, joined with a hematologist and leukemia specialist, Ernest McCulloch, to search for a way to quantify the radiation-induced loss of normal cells versus cancer cells to judge how the field of radiation therapy could be guided. They chose to rescue irradiated mice with graded numbers of bone marrow cells, then planned to see a radiation dose-dependent reduction of donor marrow-driven host reconstitution. Amazingly, they autopsied mice in the groups that had received less than fully radioprotective numbers of marrow cells and noticed bumps on their spleens. Unlike most investigators, instead of throwing the mice away, they showed that every bump contained a heterogeneous set of blood cells of the granulocyte, monocyte/macrophage, red cell, and megakaryocyte lineages, and they showed also that the number of colonies seen at days 8 or 10 after transplant was linearly related to the number of cells injected (6). I have written elsewhere about the day I read the paper (7).

The team assembled by Till and McCulloch rapidly sought to test whether each colony was clonal or derived from distinct progenitors that cohabited in the spleen, and they also tested whether the colony-forming cells could be regenerated in the colony, that is, could be self-renewed. To test the clonality of the colonies, Becker et al. devised a most clever method, simple and beautiful in concept and execution. They knew that radiation could cause DNA strand breaks, most of which were lethal, but sometimes were repaired in a way such that unique cell-specific chromosomal translocations, deletions, or fusions could occur. Therefore, they transferred marrow to sublethally irradiated hosts, then gave an additive dose of radiation, both to finish clearing most host cells and to chromosomally mark potential progenitors of spleen colonies. They examined 42 colonies, of which 4 had unique chromosomal markers. Nearly every metaphase from colony A had its single unique chromosomal marker, and the same was true for colonies B–D, each with their own unique chromosomal marker. Four more clones of the same type were reported in a note added in proof. Although the numbers were not great, the results were conclusive: every spleen colony that could be examined was clonal, and therefore at least myeloerythroid cells had a common precursor (1).

The same year Siminovitch and the team (8) showed that some spleen colonies had self-renewed spleen colony-forming cells, more in the very late, day 14 spleen colonies, and fewer at day 10. After a few years Wu, Till, Siminovitch, and McCulloch (9) used the chromosomal marking system to show that the same clone in myeloerythroid cells could also contribute to lymphocytes. This led to the concept of blood-forming, or hematopoietic stem cells (HSCs), and their later prospective isolation (10). Isolated HSCs could only give rise to day 12–14 spleen colonies in irradiated hosts, but so could prospectively isolated transient amplifying but not perpetually self-renewing multipotent progenitors. Even more differentiated cells, the common myeloid progenitors and megakaryocyte/erythroid progenitors, were largely responsible for day 8 and day 10 spleen colonies (11).
There are some curiosities concerning these publications. Till, McCulloch, Becker, and Siminovitch were curiously silent on the concept of HSCs until after these seminal papers were published. The initial papers were mainly published in specialty journals, not the “big three.” The data were not extensive, although they led to enduring and reproducible results, which contrasts to current publications that seem to need to be three to four papers packaged into one to be published.

But what is clear is that Becker, Till, and McCulloch established the principles of what might be considered clonal lineage tracing of cells in vivo, and they did so in a convincing and elegant manner that current investigators would be well advised to emulate.

Disclosures
The author has no financial conflicts of interest.

References