PERSPECTIVE

Hematopoietic stem cells depend on HIM and HER

David G. Kent*

York Biomedical Research Institute, Department of Biology, University of York, York, United Kingdom

After a series of pioneering experiments on hematopoietic stem cell (HSC) function, including early evidence of heterogeneous stem cell behavior, Jim Till, Ernest McCulloch, and Lou Simminovitch put forward a stochastic model for stem cell proliferation. In contrast to the alternative model, called the "hemopoietic-inductive microenvironment" ("HIM") in which specific microenvironments drove specific and consistent outcomes, they coined the term "hemopoiesis engendered randomly" ("HER"), in which HSCs have intrinsic differences in the cellular state that introduce a probability of potential outcomes. The HIM (extrinsic) and HER (intrinsic) discussion continues nearly 60 years later, but the metaphor also has relevance beyond cellular decision making if one considers the infrastructure and systems supporting the actual scientists who make these advances—a different kind of HER and HIM, but no less important to sort out. This article concludes with some thoughts on how we might achieve a better balance between the HIMs and HERs undertaking the research as well.

HIGHLIGHTS

- Intrinsic and extrinsic regulators of hematopoietic stem cells have been investigated for more than six decades.
- The "hemopoietic-inductive microenvironment" ("HIM") is when specific microenvironments drive specific and consistent outcomes.
- "Hemopoiesis engendered randomly" ("HER") is when hematopoietic stem cells have intrinsic differences in the cellular state that introduce a probability of potential outcomes.
- The people undertaking the research (a different set of "HIMs" and "HERs") also warrant consideration when thinking about stem cell research.

THE HISTORY OF "HEMOPOIESIS ENGENDERED RANDOMLY" AND "HEMOPOIETIC-INDUCTIVE MICROENVIRONMENT"

After the discovery of discrete clonal units of blood-forming cells (the spleen colony assay or "CFU-S"), the race was on to figure out the conceptual and experimental properties of these potent single cells. Although it was not named "hemopoiesis engendered randomly" ("HER") at the time, the stochastic model was first presented in a 1964 *Proceedings of the National Academy of Sciences* article by Till, McCulloch, and Simminovitch [1]. This was the first introduction of mathematical modeling to hematopoietic stem cell (HSC) biology in which a simple "birth" and "death" framework was set up to describe the creation of progeny from a CFU-S—birth being the creation of new CFU-S, and death being progeny that were no longer CFU-S. The frequency, timing, and rigidity of "birth" decisions, however, were less clear. The hypothesis put forward in this review was that this process could be a random probability rather than a fixed preordained cell-by-cell process (or an inherited process from its parent) and used Monte Carlo simulations in parallel with their experimental observations to illustrate how this could work.

The original article read: *"Consider as an example the case in which the birth and death probabilities are arbitrarily set equal to 0.6 and 0.4, respectively. Let the six digits 0, 1, 2, 3, 4, and 5 signify a "birth," and the four digits 6, 7, 8, and 9 signify a "death." If the first random number to be drawn was a 5, then the model would show a birth, or an increase from one cell to two cells."*

They then basically "rolled the dice" (i.e., where the Monte Carlo name comes from) to see how well the experimental data would fit and demonstrated that probabilities could explain the diversity and heterogeneity observed in CFU-S assays. Of course, there were many assumptions about fixed generation time and fixed probabilities throughout the experimental duration, but the article established a potential framework to start from and, importantly, ended with a speculative note about the need to determine factors that could alter these probabilities.

This is where the work of Curry, Trentin, and Wolf came to the fore in their 1967 *Journal of Experimental Medicine* article [2] on red cell colony formation. They proposed that the environment in which an HSC found itself could create a "hemopoietic-inductive microenvironment," or "HIM" and that the presence or absence of such factor (s) would dictate the type of progeny created by a stem cell. In an elegant series of colony assays performed from different settings with or without erythropoietin (EPO) stimulation in irradiated and nonirradiated mice, they showed that erythroid colonies were formed where both EPO and an "erythroid HIM" were present, but that EPO alone would not drive red cell differentiation and that cells put into a "granuloid HIM" would instead make granulocytes. They end by

Offprint requests to: David G Kent, York Biomedical Research Institute, Department of Biology, University of York, York, YO10 5NG, United Kingdom; E-mail: david.kent@york.ac.uk.

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suggesting that the increased erythroid HIM in the spleen, compared with that in the marrow, which is richer in granuloid HIM, might explain the distinct cell types present in each tissue.

A simple way to unify things would be to suggest that all CFU-S (and HSCs for that matter) are created equal and the environment provided an instructive signal that would tell this blank canvas how to paint itself. However, one aspect of the HER model that would seemingly lay dormant for decades was that clone-to-clone variations could also result from stochastic events intrinsic to the cell, perhaps genetic or epigenetic, which could also occur with a measurable probability even in the absence of "instructive" environmental influences [3]—questions that require new tools to be resolved.

ADDING TO THE PUZZLE—THE W AND STEEL MICE

Around the same time, a series of experiments with mouse models were being performed that presented similar conundrums in the intrinsic versus extrinsic driver debate [4,5]. The sets of mice were mutant at the *W* locus (called the dominant spotting locus) and the *Steel* locus (named for its diluted-looking coat color phenotype), in particular the Steel Dickie mouse (Sl^d), which was later shown to lack a transmembrane version of stem cell factor, the gene product of the locus [6]. Both the W mice and the Sl^d have similar phenotypic traits, including mild anemia and increased sensitivity to irradiation. However, when transplantation, blood phenotyping, and colony-forming assays were undertaken, a curious phenomenon was revealed—whereas transplantation of wild-type cells could rescue W mutants, they could not rescue Sl^d mutants. Even more curiously, transplantation of Sl^d cells into W mutants could also rescue the phenotype, suggesting that there was nothing wrong with the input Sl^d blood cells. Much later, molecular evidence revealed that this was because c-Kit (coded by the W locus) and SCF (coded by the Sl locus) were a ligand–receptor pair and the reason why Sl^d cells could rescue a W phenotype is because the nonhematopoietic cells of that W-mutant recipient animal had intact SCF signaling as a result of the microenvironmental cells (not the blood cells) that expressed SCF, but they simply could not signal effectively to the W-mutant blood cells. These genetic mouse model data provided some of the first early evidence that a factor extrinsic to the colony-forming cells themselves could drive such dramatic system-wide phenotypes, and it was the result of having robust assays and tools in place that allowed trackable phenotypes to be studied accurately.

Early victory and general scientific research efforts gravitated toward the extrinsic model with concepts such as "HIM" being simpler and more definable, but this simplicity does not mean that the HIM model is correct or the only way to view the system, and sometimes it takes a long time for new approaches to be explored.

FAST FORWARD TO 2022—PROBABILITIES AND CONTINUUMS

A huge amount of evidence, over the last decade, in particular, has resolved two things in the field. First, all HSCs are not equal—neither at the cellular (reviewed in Copley et al. [7]) nor at the molecular (reviewed in Laurenti and Göttgens [8]) level. Second, hematopoietic stem and progenitor cells (HSPCs) of all types do not exist in definable boxes of known potential, and the entire hierarchy of differentiation cascades is more regularly being viewed as a continuum of probabilities (remember that "HER" model?) (reviewed in Laurenti and Göttgens [8]). Single-cell transcriptional profiling, lineage tracing, and cellular barcoding efforts, in particular [9–15], although numerous other -omics datasets have reinforced this (reviewed in Bode et al. [16]), have focused people's attention on global molecular states being far more fluid than the rigid programs invoked by master regulators or licensing factors. Although the cell-to-cell noise created by sampling variability almost certainly contributes to these datasets being viewed as heterogeneous, there are clear transition points that help shape the cellular identity of the progeny of a particular HSPC, and these are being studied extensively. The debate continues as we try to make sense of these probabilities and the impact of specific instructive signals. Central to this debate is the generation of new tools to monitor gene products more accurately (such as the "activity" reported by Wang et al. [17]) and the search for factors that alter the probabilities within a standard hierarchic differentiation cascade. At present though, it seems reasonable to think that a contribution from both intrinsic and extrinsic regulators will be involved.

THE NEXT LEVEL OF HIM AND HER

In closing this Perspective piece, I would be remiss not to mention the other large HIM versus HER debate that is impacting our ability to undertake scientific research, namely, the structure of the scientific workforce—although it is worth stressing that the HIM/HER analogy should not stretch to data interpretation. As a discipline, biomedical sciences recently has had a reasonable balance between men and women undertaking research. This is particularly strong at the early stages of training (Ph.D. and postdoctoral fellow), although it notably drops off as the seniority level climbs, with Professor positions occupied by women regularly being less than 25% across institutions, fields, and countries. This sort of structure does not mean that men and women are undertaking grossly disproportionate amounts of research, but it does mean that the credit and recognition are not always fairly apportioned.

My own experience and progress in the field of experimental hematology have been substantially shaped by women scientists, and some interesting observations have emerged. From my Ph.D. supervisor (Professor Connie Eaves) through to collaborations in my postdoctoral training and as a group leader, women scientists have been absolutely essential partners in my research journey, but the structures created over time in research do not recognize all types of contributions equally. We do not achieve much as scientists without top-rated infrastructure and people who support us, and very few studies are driven by a single individual. When I look back at the laboratories I have interacted with along the way, it was not uncommon to have a Ph.D.-holding staff scientist (or long-term postdoc) carrying the weight of the laboratory—and this researcher is regularly a woman, although many men also find themselves in this type of post. The scientific engine and experiential encyclopedia of the laboratory, this person is regularly afflicted by salary ceilings and short-term contracts, often operating in the shadow of the laboratory head. If the laboratory head decides to trot off to another institution or retire, the institution rarely has a good career plan for the laboratory lieutenant. The solution is to better recognize and reward these critical positions and to widen participation at all levels—but these are frustratingly slow roads to travel down.

ISEH LEADERSHIP AND THE FUTURE OF HIM AND HER

A quick look through the list of former ISEH presidents gives a glimpse of how things have been changing—the first 31 ISEH

presidents were men and the first eight editors of *Experimental Hematology* were men. More recently, nine of 21 (43%) presidents have been women, 50% of editors at *Experimental Hematology* are women, and the ISEH Board is >50% women. For me (someone attending ISEH since 2005), leadership by women has been a real strength of the society, especially in the area of developmental HSC biology where our scientific leaders are predominantly women, and have been for decades. Embedded within the ISEH community, however, are a large number of laboratory lieutenants, staff scientists, senior postdoctoral fellows, technicians, and others who keep the engine of discovery purring along—they are not the "leaders" displayed in photos or recognized by awards, but they are most certainly leading the charge for passionate discovery-based science and rigorous standards in the community and we need to do better by them by changing the structures that support scientists to allow for both HIM and HER to be a valued part of HSC research going forward.

WE NEED TO DO BETTER

The structures that fund science need to change and the senior people who hire, promote, and work to retain researchers need to gain a better understanding of how the research is performed and who is essential to it humming along. However, these agencies and senior people are not going to change on their own, and scientists need to drive these actions themselves so that their own future research culture improves. Efforts are underway in the global early career researcher community [18], but fields that care about the integrity of their own space need to mobilize their societies to do bigger and better.

Conflict of Interest

The author has no conflicts of interest to declare in relation to this work.

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