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The GOD of Hematopoietic Stem Cells:

A Clonal Diversity Model of the Stem Cell Compartment

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Abstract

Hematopoietic stem cells (HSC) show heterogeneous behavior even when isolated as phenotypically homogeneous populations. The cellular and molecular mechanisms that control the generation of diversity (GOD) in the HSC compartment are not well understood, but have been the focus of much debate. There is increasing evidence that the most important HSC functions, self-renewal and differentiation, are epigenetically preprogrammed and therefore predictable. Indeed, recent data show that the adult HSC compartment consists of a limited number of functionally distinct subsets of HSC. This contradicts older models of HSC behavior, which postulated a single type of HSC that can be continuously molded into different subtypes of HSC. We propose a clonal diversity model where the adult HSC compartment consists of a fixed number of different types of HSC, each with epigenetically preprogrammed behavior. Aging or disease may change the overall function of the HSC population. The model predicts that these changes reflect the relative composition of the HSC subsets, rather than changes in individual HSC. This view has implications for using HSC in experimental and clinical settings. Selection for the appropriate subsets of HSC, rather than attempts to force HSC to adjust, should improve their utility in transplantation and gene transfer applications.

Keywords

hematopoietic stem cells; stem cell purification; stem cell subsets; deterministic; stochastic; epigenetic; lineage-bias; IL-7; niche; generation of diversity; aging

HETEROGENEITY IN THE HSC COMPARTMENT

Hematopoietic stem cells (HSC) differentiate to replenish all cells of all of the hematopoietic lineages, including lymphocytes, myeloid cells, and erythrocytes. Upon commitment to differentiation, HSC are thought to divide and generate one each of a lymphoid and a myeloid restricted progenitor. Each of these progenitors will then differentiate into precursors that have fewer and fewer lineage choices. Finally, unipotential precursors will give rise to mature cells. The astounding demand for mature cells in the periphery (more than 10^{11} leukocytes are generated every day in humans) creates a constant pressure on HSC to provide differentiated progeny. Yet, when HSC commit to differentiation, they lose self-renewal capacity and pluripotency and cease being HSC. To avoid depleting the HSC compartment, HSC will proliferate to generate more HSC. Thus, self-renewal and pluripotent differentiation capacity together are defining characteristics of HSC. As all adult stem cells, HSC are tissue specific: a HSC will give rise only extremely infrequently to cells of the nonhematopoietic lineages.[1]

The hematopoietic differentiation process accounts for most of the extensive heterogeneity of the hematopoietic tissues. Heterogeneity has also been demonstrated in the HSC compartment

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itself. HSC can differ in how rapidly they repopulate an ablated host after transplantation, how long they can produce mature cells, and how much they can self-renew.[2-16] The molecular and cellular mechanisms that generate such diversity in the HSC compartment have been much debated, but have been difficult to assess experimentally.

Many theories have been developed to explain the generation of diversity (GOD) in the HSC compartment. The major themes of these theories are intrinsic vs. instructive. The instructive models,[17-19] postulate that the microenvironment educates HSC to change. The models of intrinsic regulation of HSC diversity can be subdivided into stochastic and deterministic theories. These hypothesize respectively that HSC behavior is either not predictable (stochastic) or predictable (deterministic).[20-26] For a comprehensive overview of models see the (review by Viswanathan and Zandstra; ref. [27]). Without fail, all currently accepted theories assume the existence of a single type of mother-of-all-HSC (or most primitive HSC) in adult bone marrow (BM). Such HSC will continuously generate diversity by producing functionally heterogeneous daughter HSC. Data from our group challenge this concept. We summarize evidence that the HSC compartment, at least in the adult mouse, consists of a limited number of HSC types—each with predictable behavior. Based on these data, we sketch a model of GOD for HSC in a deterministic system.

THE BOLTS AND NUTS OF DETECTING HSC

Most of the properties of a HSC can be detected in vivo in transplantation assays.[3] HSC will find all signals necessary for self-renewal and differentiation in the host environment. The injected HSC and the ablated hosts are chosen from strains of mice that are congenic for easily distinguishable markers such as the cell surface antigen CD45 or the intracellular transgene green fluorescent protein. Mature cells derived from the injected (donor type) HSC therefore can be enumerated by immunofluorescence in a few drops of blood. The white blood cells are also stained with markers specific for the lymphocyte or myeloid-erythroid lineages to ascertain that the injected cell is pluripotent, i.e., gives rise to all hematopoietic lineages. Because this assay requires little blood, hosts can be tested repeatedly and the kinetics with which HSC repopulate the periphery with differentiated cells can be measured. For the studies described here, criteria were chosen that allowed the detection of HSC but excluded non-HSC (progenitors and precursors) from the analysis.

Whether a HSC had self-renewed can be measured by serial transplantation of the donor type HSC, by injecting these into cohorts of secondary, tertiary and quaternary hosts. If the secondary hosts are repopulated by donor type cells, one can conclude that the original donor HSC had self-renewed in the primary host. As an aside, methods other than transplantation are notoriously unreliable for the detection of HSC that had been transplanted previously.[28,29] Overall, repopulation patterns are highly informative for HSC function and the functional diversity of HSC is resolvable through the behavior of clonal HSC in repopulation experiments.

Why is it necessary to perform clonal analysis of HSC behavior? Decisions about self-renewal and differentiation are made on the level of a single HSC. If one tests many HSC at the same time, one will inevitably detect the composite behavior of the HSC in the pool. The contribution of individual HSC will be obscured, making it difficult to derive meaningful information on the GOD of HSC. Our group used limiting dilution strategies together with streamlined experimental and statistical approaches to examine HSC on the clonal level. This approach allowed us to examine a large number of clonally derived HSC.[2-4,30-32] So far we have followed the repopulation behavior of 106 individual HSC clones for at least seven months. The data provided strong evidence that much of the behavior of HSC is predetermined.

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HOW MANY DIFFERENT TYPES OF HSC EXIST IN THE HSC COMPARTMENT?

We asked whether our set of clonal HSC contained all possible patterns of repopulation kinetics.[3] Since donor type cells were enumerated every other month for at least seven months, each repopulation curve was comprised of at least three segments. The individual segments were symbolized so that a positive slope was labeled with a "+", a negative with a "−" and a flat slope as "~". The symbolized repopulation curves were then compared to each other and sorted into groups if they were of identical shape.[31] We used the relative Hamming distance,[33] a method from quantum mechanics, to compare the shapes of the symbolized curves.[3,31] Interestingly, less than 30% of all possible kinetics were found and there is a vanishingly low probability that the analysis of additional HSC would yield new types of repopulation curves.[3] The most likely interpretation of this finding is that the HSC compartment consists of a limited number of different subsets of HSC.

Previous models of GOD in the HSC compartment predicted that HSC would constantly create new heterogeneity. However, this is not the case. We found that individual HSC do not regenerate the heterogeneity seen in the HSC compartment. Rather, individual HSC self-renew to give rise to daughter HSC which are very similar to each other in their kinetics of repopulation. Moreover, the daughter HSC gave rise to mature cells with the same lineage ratios.[4] Thus, HSC in the adult can be classified into a small number of HSC types, each with predetermined and predictable proliferation and differentiation abilities.

To reveal the full potential of the compartment, HSC clones were obtained from unseparated BM. Next, we asked whether different purification methods would enrich similarly for all subsets of HSC. We compared the clonal repopulation curves from two populations of HSC with a different, albeit partially overlapping phenotype (Fig. 1). HSC enriched as $Lineage^{-}Rhodamine^{low}$ Side Population cells were sorted and tested in Vancouver and the data have been published previously.[3,34] Lineage-Sca-1+ckit+ (LSK) cells were sorted in our laboratory and injected in limiting numbers into ablated hosts (unpublished). All repopulation curves derived from both populations of sorted cells fit into the groups defined already by HSC from unseparated BM and no new kinetics were identified. Thus, the HSC classifications are remarkably robust, since they held true when data from two different laboratories, generated with three different methods were compared. Interestingly, the purification methods appear to select for different, albeit overlapping, subsets of HSC (Fig. 1). For example, the LSK cells do not generate repopulation curves with steadily declining levels of donor type cells (− − −). In contrast, Rho^{low} cells are enriched for such − − − kinetics.[3] Relatively few repopulation curves were examined, suggesting that additional analysis might fill in some of the gaps (Fig. 1). Yet, it is unlikely that overall distribution will change much. Thus, different purification methods can unexpectedly enrich very different subsets of HSC.

Since the subsets of HSC differ noticeably in function, it would not be surprising if each had a different expressed gene program. Indeed, there is evidence that the subsets of short-term and long-term repopulating HSC differ in that respect.[35] Thus, it will be important to define the clonal composition of different populations of HSC that are obtained with different selection methods. Perhaps, a different composition of HSC types in different purified populations could reconcile some of the strikingly discrepant results in HSC research. For example, when different groups catalogued the expressed gene program of HSC there was little overlap in the genes identified.[36-38]

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LINEAGE-BIASED HSC—A PARADIGM OF EPIGENETICALLY FIXED HSC BEHAVIOR

Another unexpected finding from the clonal analysis of HSC was the discovery of lineage-biased HSC.[2,4] All HSC can be classified by the ratio of lymphoid to myeloid cells that they generate upon differentiation. Three classes of HSC have been defined: balanced HSC that repopulate peripheral white blood cells in the same ratio of myeloid to lymphoid cells as seen in unmanipulated mice (about 15% myeloid and 85% lymphocytes). Myeloid-biased (My-bi) HSC give rise to too few lymphocytes, and lymphoid-biased (Ly-bi) HSC generate too few myeloid cells.[2] All three types are normal HSC in that they have self-renewal capacity and can regenerate all hematopoietic lineages (pluripotency). Strikingly, the lineage bias is preserved through multiple rounds of serial transplantation: balanced HSC self-renew to give rise to daughter HSC that are also balanced, My-bi HSC give rise to My-bi daughter HSC, and Ly-bi produce Ly-bi daughter HSC. There is no precursor-progeny relationship between the three types of HSC and they do not represent stages of differentiation. Rather, these are three classes of HSC, each with an epigenetically fixed differentiation program.

How could epigenetic imprinting on the level of the HSC affect the generation of downstream differentiated progeny? My-bi HSC were studied in more detail to define the mechanism(s) that account for the lineage bias. We found that My-bi HSC give rise to normal myeloid progeny but their lymphoid progeny showed a blunted response to Interleukin-7.[2] This lymphokine is central for the differentiation and homeostatic proliferation of lymphoid cells.[39,40] Analogously, Ly-bi HSC have an impaired ability to generate myeloid progeny.[2] It is likely that each type of HSC expresses a different epigenetically fixed set of genes that changes downstream expression of genes necessary for differentiation.

We reexamined the groups of HSC classified according to their repopulation kinetics[3] and found that balanced, My-bi and Ly-bi HSC tend to segregate into different repopulation groups although there is some overlap (unpublished). This agrees with the observation that the classification of the kinetics predicted to some extent self-renewal capacity.[3] For example, kinetics with a steady increase (+ + +) were more likely to self-renew than HSC with other kinetics. Similarly, My-bi HSC generally have more self-renewal capacity than other types of HSC and + + + kinetics are the most frequent repopulation patterns for My-bi HSC.[2] Thus, both self-renewal and differentiation behavior of individual HSC is predictable and therefore preprogrammed on the level of the HSC. If adult HSC are capable of generating heterogeneous daughter HSC, this must be a very infrequent event.

A CLONAL DIVERSITY MODEL OF THE GOD OF HSC

Our data are not consistent with the older models of how heterogeneity is generated in the HSC compartment. The best fit for the data is a model where the adult HSC compartment consists of functionally discrete subsets of HSC, and the GOD is largely complete. Since many HSC are active at any given time point,[5,6,41,42] the steady state hematopoietic system will be derived from the combined functions of these different types of HSC. However, changes in the HSC compartment can be induced—for example through diseases, chemotherapy, transplantation, cytokine treatment, and aging.[43,34,44-53] After such events the HSC compartment can become pauciclonal.

Our model predicts that such functional changes of the HSC population are caused by the depletion of some subsets of HSC with the concurrent enrichment in other subsets. Rather than changing the function of individual HSC as has been proposed by previous hypotheses, we predict a shift in the composition of the HSC subsets. One way this might be achieved is if different types of HSC would be more sensitive to hematological toxicity than other types.

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Alternatively, depletion or enrichment of HSC could come from the different life spans of the HSC.[2] Short-lived HSC could simply be depleted for and long-lived HSC may be enriched after hematological stress or aging. Indeed, preliminary data from our laboratory suggest that in aged BM, Ly-bi HSC are depleted while My-bi HSC are enriched (unpublished). An HSC compartment depleted of the rapidly repopulating Ly-bi could account, at least in part, for the defects in immunity seen in the aged. The clonal diversity model could also explain why increases in the number of HSC are beneficial for transplantation patients. The higher the dose of HSC transplanted the greater the probability that all types of HSC will be replenished to create a healthy diversity of the HSC compartment in the host.

The lack of heterogeneity in the daughter HSC after clonal self-renewal[2,4] indicates that in vivo niches do not play a role in the GOD. Thus, the microenvironment is permissive, rather than instructive. The clonal diversity model makes the prediction that extrinsic signals can modulate predetermined HSC behaviors, but cannot change them fundamentally. For example, a putative factor that supports HSC self-renewal might enhance the self-renewal divisions of a HSC that is already primed to self-renew. However, that same factor will not help a HSC that is not in self-renewal mode.

Epigenetic imprinting of the expressed gene program would provide a molecular basis for deterministic HSC behavior. The dynamical changes in the epigenome during differentiation are increasingly well understood.[54] HSC (analyzed as a population) show open chromatin configurations for lineage specific genes and HSC seem to express lineage specific genes promiscuously.[54-57] Differentiation is accompanied by the expression of genes, such as Pax5, that suppress genes needed for the development into other lineages.[58] There is evidence that removing or interfering with epigenetic imprinting will protect HSC maintenance, even if the culture conditions favor differentiation.[59] This is consistent with the interpretation that differentiation causes restrictions of a multipotent gene program. Nevertheless, it is unclear whether the “promiscuity” is derived from the heterogeneity of the analyzed population rather than the indiscriminate gene expression pattern of a single type of HSC.

The epigenetic fixation of HSC described here must be more stable and less dynamic than the processes involved in differentiation. At the same time, it is likely that different gene networks are responsible for differentiation and epigenetic fixation of HSC function. The preprogrammed lineage potential of HSC is likely a reflection of an epigenetically fixed set of genes that interferes with the expression of genes necessary for a robust response to lineage specific signals in the progeny of the HSC. The expression patterns of such a set of genes are “inherited” to all daughter HSC. In contrast, differentiation associated changes in the epigenome are not maintained on the HSC level. Thus, the epigenetic events that control HSC behavior are likely upstream of those that control differentiation.

An obvious extension of the clonal diversity model is that epigenetic imprinting must have happened during development. In analogy to the earliest differentiation events in the fertilized oocyte and embryonic stem cells,[60,61] HSC in early development might be unrestricted in their (hematopoietic) gene program. This would allow maximal adaptation to the rapidly changing environments when the developing HSC migrate from the aorta-gonadmesonephros (AGM) to the fetal liver to the BM during embryonic and fetal development.[62,63] HSC might become increasingly epigenetically restricted during development, concurrently with a lessening of the demand for proliferation to fill the growing hematopoietic tissues. There is evidence that the frequency of preleukemic clones is much higher in newborns than the corresponding disease in children.[64] It is tempting to speculate that restricting the potential of HSC in the adult might also limit the survival of preleukemic HSC clones and thus protect against full leukemic transformation.[65]

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The model that the adult HSC compartment consists of a mixture of preprogrammed HSC has some immediate implications for the clinical application of HSC. It is not unreasonable to assume that different applications would benefit from using different types of HSC. For example, one might consider selectively transplanting Ly-bi HSC for a severe case of lymphopenia. Similarly, an HSC graft for a gene therapy application would benefit from preselecting long-lived HSC. This would increase the efficiency of gene transfer specifically into long-lived HSC and would assure a life-long supply of HSC carrying the transgene. In contrast, it is essential that rapidly repopulating HSC are present in HSC transplants given as supportive therapy after marrow ablative treatment. So far, attempts to tailor HSC to specific applications have been based on the concept that HSC can be molded to the application. For example, cytokine cocktails are used to try to manipulate HSC ex vivo. Perhaps the limited success of such approaches is the strongest support for a clonal diversity model and the idea that HSC should be selected according to their preprogrammed potential to fit the application.

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ABBREVIATIONS

HSC, hematopoietic stem cells; GOD, generation of diversity; BM, bone marrow; My-bi, myeloid-biased; Ly-bi, lymphoid biased; IL-7, interleukin-7.

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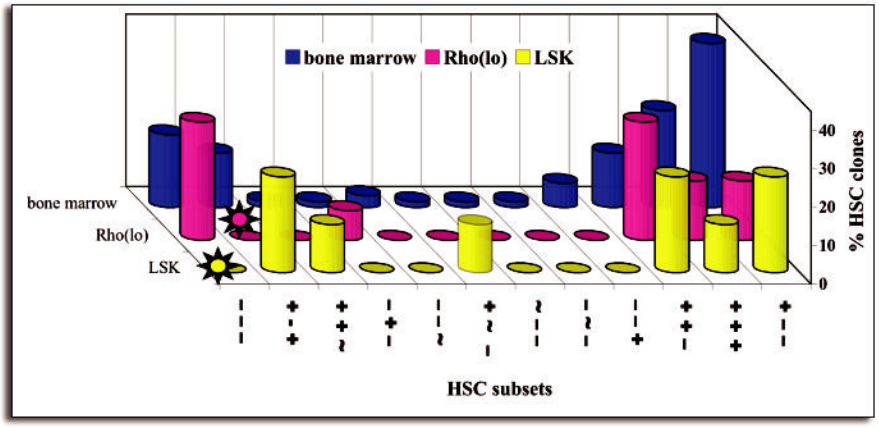

Figure 1.

Different purification protocols enrich for different subsets of HSC (modified from ref. [2]) Clonally derived HSC were classified according to their kinetics of repopulation as described. [3,31] These represent distinct subsets of HSC (indicated on horizontal axis). The data are expressed as percent of HSC types found in each of the subsets. Three populations were tested: unseparated bone marrow (dark blue), or HSC purified as lineage^{-} Rhodamine-123^{low} Hoechst 33342^{-} side population cells (Rho$^{(low)}$, shown in red) or Lin-Sca-1+ckit+ (LSK; shown in yellow). Bone marrow encompasses individual repopulation kinetics of 84 HSC, Rho$^{(low)}$ had 12, and LSK had 10 kinetics. Stars highlight the missing − − − subset in the LSK population and the missing + − + subset in the Ro(lo) population.