inside blood commentary

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HEMATOPOIESIS AND STEM CELLS

Comment on Nestorowa et al, page e20

Hematopoiesis at single-cell resolution

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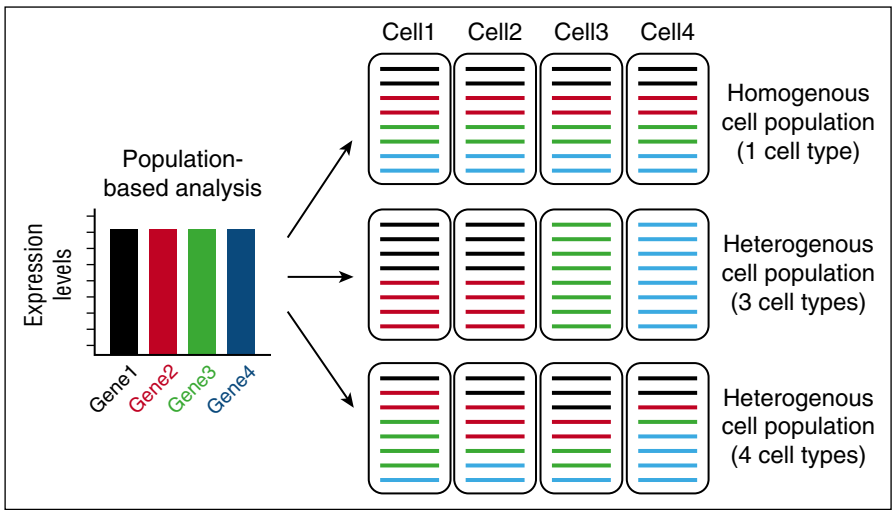
In this issue of *Blood*, Nestorowa et al present an extensive single-cell gene expression catalog of mouse hematopoietic stem and progenitor cells and use these data to reveal the early developmental trajectories that underlie the formation of mature blood cells.[1]

A major aim in biomedical research is to detangle the complexity of systems that are made up of individual cells. Such work includes identification of the cells that underlie one or more defined biological responses and establishment of the developmental pathways that lead to production of the mature functional effector cells. In preclinical research, the possibility to isolate cells at defined stages of development provides researchers with the necessary tools to directly study the regulation of differentiation. This enhanced understanding of differentiation allows us to define what happens with erroneous regulation at specific stages of development.

To understand hematopoiesis, the prospective identification, isolation, and functional characterization of candidate cell populations obtained using flow cytometric cell sorting have been a mainstay for the last 30 years. Such work has revealed that differentiation of hematopoietic stem cells into 1 or more of the mature effector cells of the blood involve several intermediate developmental stages. The current view of hematopoiesis therefore describes defined progenitor populations and their relationships to each other.[2] There are, however, inherent limitations associated with population-based approaches, which may explain many of the controversies in this area. Given the potency of many primitive hematopoietic cell types in terms of proliferation and/or self-renewal, exemplified for instance by the ability of even single hematopoietic stem cells to be able to multilineage repopulate a new host,[3] the purity of obtained populations is critical. Impurities in this regard may not be "technical failures" but rather due to the heterogeneous expression patterns of surface markers at the snapshot time of analysis/cell isolation.



The detection of expressed genes in a population-based measurement (left) can be the result of many possible scenarios (right): all cells might have an equal amount of the transcripts (top), some cells might express a given gene while others do not (middle), and/or there can be grades of expression between different cells (bottom). Direct analysis of single cells, as approached by Nestorowa et al, is the only way to deconvolute which of these scenarios are present.

Imbued within the concerns of impurity is also the unlikeliness that any given cell population is homogenous in its strictest sense. Thus, although most research in cell biology has historically been conducted at population levels, it is an undisputed fact that such averaged approaches cannot distinguish between the characteristics and responses of the individual cells that make up a population of cells (see figure). Therefore, deviations are to be expected based on the residence time a cell spends in a given cellular compartment, which introduces proliferation history and position in the cell cycle at the time of investigation as potentially confounding factors.

Nestorowa et al use murine hematopoiesis as a model to provide a framework for how many of these issues can be resolved. The approach rests on the general assumption that gene expression might represent an unbiased approach to reveal the relationship between cells.[4] Although this is a quite well-established assumption that has been successfully applied to other cellular systems, the murine hematopoietic system is rather unique in that a vast amount of information is already available on cell surface markers that can be correlated to cellular function.[2] Thus, with an ambition

to intersect this information in an unbiased manner, the authors took advantage of the possibility to index every cell evaluated based on a set of flow cytometric (marker) properties,[5] but avoiding the pitfalls that associate with standard cell sorting, where manual decisions have to be made as to what constitutes positivity and negativity for each marker. Of note, the index approach represents a considerable advantage currently unattainable by many alternative high-throughput single-cell gene expression approaches such as Drop-Seq[6] or the use of automated preparation platforms such as the Fluidigm C1. Using this single-cell approach, subsequent analyses revealed not only the transcriptional relationships among all evaluated cells but also to what degree known cell surface markers associate with these relationships.

Although many of the key findings in the work provide supporting evidence to already existing models of hematopoietic differentiation, the work also provides a rather clear guide as to the purity of previously described progenitor populations. Hence, the data serve as a good starting point when reevaluating work that emanated from a time when these sophisticated methods were not available. Furthermore, as exemplified in the paper, the data constitute a relevant reference point for intersections of other single-cell gene expression datasets. Given the wide availability of different types of transgenic mice, including highly defined models of leukemia, it is therefore easy to foresee its widespread utility for the broader scientific community. Also of note is the interactive tool located at http://blood.stemcells.cam.ac.uk/single_cell_atlas.html, which accompanies the paper, where users can themselves mine their own favorite gene(s) throughout the identified differentiation trajectories.

In conclusion, the approaches taken by Nestorowa et al draw on the recent developments of genome-wide single-cell transcriptional approaches as a potential solution to define the earliest stages of hematopoietic development. Apart from being particularly relevant to understand rare cell types such as hematopoietic stem cells, which can otherwise drown in the noise derived from contaminating cells, approaching hematopoiesis from a single-cell perspective is also highly relevant for disease, where pathologic states such as leukemia emanate as a consequence of deregulation of an individual cell.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Alvarnas et al, page 1050

A new standard for HIV-associated lymphoma

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In this issue of *Blood*, Alvarnas et al report a prospective multicenter clinical trial demonstrating that autologous hematopoietic cell transplantation (AHCT) can be performed safely and effectively in patients with HIV-associated lymphomas, with success rates comparable to those in the HIV-negative population.[1]

When HIV/AIDS was first identified in the early 1980s, the diagnosis was nearly universally fatal, given the lack of effective treatments. The transformation of HIV/AIDS from a virtual death sentence into a survivable chronic illness is a major achievement of modern biomedical science, driven by patient and community activism. People with HIV/AIDS have long been recognized to have an increased risk of numerous malignancies, including both non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL). Although modern combination antiretroviral therapy has significantly decreased the incidence of HIV-associated NHL, the incidence remains higher than among HIV-negative individuals.[2] In contrast, the incidence of HL appears to have increased somewhat since the advent of modern antiretroviral therapy.[3]

People with HIV-associated lymphoma can be treated effectively with combination chemotherapy induction regimens, resulting in progression-free survival (PFS) in approximately two-thirds of patients at 2 years.[4] However, for people with refractory or relapsed HIV-associated lymphoma, the optimal treatment has been unclear. In the HIV-negative population, such patients are routinely offered high-dose chemotherapy with AHCT as the most effective and potentially curative treatment. Although case reports and 1 prospective study have reported the use of AHCT in HIV-associated lymphoma,[5] HIV is considered an exclusion for most transplant-based clinical trials, and AHCT in HIV-positive patients has generally been restricted to centers with dedicated expertise, due to concerns about the management of antiretroviral therapy, drug-drug interactions, and a perceived increase in the risk of regimen-related toxicity and infections in the HIV-positive population. Thus, widespread access to this potentially curative therapy for people with relapsed or refractory HIV-associated lymphoma remains a major unmet clinical need, driven by uncertainty over its safety and efficacy in the HIV-positive population.

To address this need, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) and the AIDS Malignancy Consortium (AMC) collaborated on a prospective multicenter phase 2 clinical trial of AHCT in HIV-associated lymphoma, with results reported in this issue of *Blood* by