Editorial

A CRISPR Take on Clonal Hematopoiesis

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Inflammation is fundamental to both atherosclerosis and the healing that occurs after myocardial infarction, but we have not yet bridged the gap between recognizing that inflammation matters and understanding exactly how the various inflammatory cascade components interact.[1] A considerable body of work on the role of inflammation in atherosclerosis and myocardial infarction has zeroed in on cytokines, chemokines, growth factors, and a dizzying repertoire of receptors, kinases, and transcription factors. This focus is well founded: in addition to being sources of great science, secreted, membrane-bound, and even intracellular proteins can be attractive therapeutic targets. Last year, the completion of CANTOS, the clinical trial that targeted the inflammatory cytokine IL (interleukin)-1β to treat cardiovascular disease, laid bare the power of such an approach.[2] Many people already receiving statins and other drugs benefited from blocking this one cytokine. Yet secreted, membrane-bound or intracellular proteins are cell products that are transcribed and translated by and for cells. And leukocytes, the white blood cells that define the immune system, are major protein producers; take a protein, even one loosely associated with the inflammatory cascade, and chances are it's leukocyte-made. We should care about leukocyte dynamics—their production, recruitment, differentiation, and function—because understanding leukocytes improves our access to the larger story involving not only cells and their products but also organs they inhabit and systems in which they participate.

Article, see p 335

The adult human bone marrow contains ≈10 000 hematopoietic stem cells (HSC) that can generate nearly all leukocytes, all red blood cells, and platelets. A few caveats notwithstanding, the general consensus, if not the default assumption, has been that any HSC is equally capable of replenishing the leukocyte pool, with no single HSC having an outsized influence on the number of leukocytes it could produce. By extension, a leukocyte population would be expected to comprise a diverse mixture randomly derived from those 10 000 HSC. However, a series of studies published over the last 4 years have argued that as we age, representational leukocyte diversity diminishes as some HSC contribute to the overall pool more than others.[3–6] The phenomenon has been called clonal hematopoiesis, and its progression correlates with cancer and coronary heart disease. (When clonal hematopoiesis occurs without additional hematologic abnormalities, it is called clonal hematopoiesis of indeterminate potential.) How exactly clonal hematopoiesis arises is not entirely known, but it is clear that acquiring certain driver mutations can endow HSC with a survival advantage, leading to their clonal expansion. Mutations in *TET2*, *DNMT3A*, *ASXL1*, and *JAK2* specifically correlate with cardiovascular disease in humans. The discovery is notable for several reasons, not the least for emphasizing that leukocyte dynamics can decisively contribute to disease progression.

How does clonal hematopoiesis aggravate cardiovascular disease? This is an important and difficult question. Recent studies have demonstrated, for example, that *Tet2*-deficient mice develop larger atherosclerotic lesions[3,5] and have accelerated heart failure.[6] As might be predicted, *Tet2*-deficient cells outcompete their wild-type counterparts and thus constitute a higher proportion of the leukocyte pool. But *Tet2*-deficient cells accelerate disease not necessarily because they are more numerous but because they produce more IL-1β and are therefore deemed more inflammatory. What, if any, is the relationship between a cell's capacity to outcompete its counterparts and its proclivity to procure inflammatory cytokines? Are those processes directly linked, distally associated, or completely unrelated? And how do mutations other than *Tet2* contribute to clonal hematopoiesis and cardiovascular disease? Answering these and other questions requires robust and easily accessible tools.

In this issue of *Circulation Research*, Sano et al[7] describe a CRISPR/Cas9 method for rapidly engineering mutated HSC to specifically study the function of Tet2 and Dnmt3a, the top 2 driver mutations implicated in clonal hematopoiesis. The approach, which is adapted from previous work,[8] involves isolating stem cells from the bone marrow of wild-type mice, transducing those cells with lentiviral vectors that contain Cas9-GFP (green fluorescent protein) and guide RNA for mutating either Tet2 or Dnmt3a, and injecting the transduced cells into a lethally-irradiated mouse. The inclusion of GFP allows for determining successful lentivirus transduction as well as successful cell engraftment in the host. The authors performed the necessary quality control experiments to ensure that the procedure worked. Because the cells of interest are bone marrow-derived, there is no need to generate a full knockout mouse, a process that would be more costly and lengthy. The procedure is not without its drawbacks: the host mice must be irradiated, and only a relatively small proportion of cells are ultimately transduced. That said, incorporating GFP facilitates clear distinction between wild-type and mutated cells, and the mixture of GFP^+ and GFP^- cells is a built-in control.

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(*Circ Res*. 2018;123:313-314.
DOI: 10.1161/CIRCRESAHA.118.313347.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.118.313347

On generating the chimeric mice, Sano et al[7] tested *Tet2* and *Dnmt3a's* role using an angiotensin II infusion cardiac dysfunction model. They confirmed that Tet2 gene disruption confers a competitive advantage to hematopoietic cells and promotes cardiac dysfunction, presumably via heightened IL-1β. They also showed, for the first time, that *Dnmt3a* gene disruption likewise promotes cardiac dysfunction. Unlike *Tet2*, however, *Dnmt3a* inactivation did not lead to leukocyte expansion, and although it did enhance inflammation, the nature of the inflammatory response differed, with higher Cxcl1 and Cxcl2 production but no significant effect on IL-1β. These observations underscore an important if not intuitively obvious point: different driver mutations will lead to different types of clones with occasionally overlapping but frequently divergent functions. It remains to be seen how Asxl1, Jak2, and other driver mutations influence clonal hematopoiesis and its sequelae.

CRISPR/Cas9 technology's impact on our ability to manipulate genes quickly cannot be overstated. However, the everyday manipulation of HSC using lentiviral vectors, as demonstrated by Sano et al,[7] is not yet certain. In order for the approach to become as routine and seemingly effortless as other standard laboratory techniques, it has to be easily accessible, accurate, and simple. To be sure, we are moving in that direction, and many laboratories already routinely use this kind of approach, but the remarkable is not yet unremarkable.

Identifying clonal hematopoiesis and its potentially causal ties to cardiovascular disease shines a spotlight on the importance of leukocytes. But analyzing how driver mutations alter a cell's capacity to produce cytokines, though essential, will not be enough to fully reveal the phenomenon's secrets. We know leukocytes are profoundly transactional and depend on each other for survival, proliferation, and function. A leukocyte repertoire arising from an assortment of stem cells might be more resilient to infection or injury by virtue of its inherently more diverse molecular toolbox; some cells, even within the same subset, will produce more IL-1β, whereas others will deliver more IL-6. A more homogeneous system might be deprived of such a balanced response, instead resorting to a potentially dangerous overproduction of fewer products. A broad inquiry into how cell diversity selects for cell behavior and, conversely, how cell behavior shapes clonal representation, perhaps even in the absence of driver mutations, is bound to be rewarding.

Disclosures

None.

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Key Words: Editorials ■ atherosclerosis ■ bone marrow ■ hematopoiesis ■ leukocytes ■ transcription factors