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INSIGHTS

Pumping blood with self-reliance and cooperation

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In this issue of *JEM*, Singhal et al. (https://doi.org/10.1084/jem.20180008) explore the cellular mechanisms involved in endothelial cell regeneration in the liver. Using a combination of myeloablative and nonmyeloablative approaches, the authors found that repair of the endothelium is mediated by endothelial cells themselves, but when injured, endothelial cells enlist myeloid counterparts that aid in vascular repair.

Much of our knowledge related to vascular growth is associated with angiogenesis, in which the expansion of the vasculature requires detachment of differentiated cells from their neighbors, invasion of the adjacent stroma, proliferation, and organization of a new vascular plexus. However, a vessel also grows in width and length after a tube has been formed. In fact, the aorta expands at least threefold from the neonatal stage until adulthood. In addition, repair of the vascular inner lining after trauma occurs in a manner that does not involve angiogenic expansion. In this case, mitosis takes place in the context of fast flow and, at times, rapid pulsatile tensional forces. How is this process accomplished? While we still do not have a full answer to this question, efforts from a number of laboratories have raised the possibility that bone marrow–derived cells could seed, incorporate, and contribute to blood vessel expansion. The evidence for this is broad. Reports have highlighted the identification of circulating cells with a strong resemblance to endothelial cells based on surface markers (Bautch, 2011; Medina et al., 2017) and showed properties in vitro that bear little distinction from differentiated endothelium. Other complementary studies found that bone marrow–derived mononuclear cells could effectively seed and aid the expansion of growing blood vessels (Iwakura et al., 2003). Combined, the data prompted the notion that perhaps endothelial cell progenitors could emerge from the bone marrow, circulate, and function to repair, regenerate, and expand vascular beds in the adult. Unfortunately, the concept was never fully accepted, as genetic and lineage-tracing experiments did not always align with the full notion that circulating cells in the adult significantly contributed to vascular growth and repair, nor supported that bone marrow was the source of endothelial progenitors (Purhonen et al., 2008). This controversy still dominates the literature and scientific meetings, but we might had taken a turn. Work by the laboratories of Augustin and Hu in this issue sheds light on this controversy, offering a path to begin the resolution of this dilemma.

The work presented by Singhal et al. used several irradiation-based myeloablative and nonmyeloablative mouse models to explore the cellular sources responsible for the regeneration of the liver vasculature. Their findings revealed the unequivocal contribution of the preexisting endothelium in the repair and expansion of the vasculature in the injured organ. Genetic tracing analysis, parabiosis experiments, and sophisticated imaging all pointed to the endothelial lineage as the sole source of new cells during vascular regeneration. In this manner, Singhal et al. (2018) stepped away from the notion that bone marrow contributed to the endothelial lining, except they also revealed that when endothelial cell division was impaired, such as during irradiation, myeloid cells were recruited by the damaged endothelium and actively contributed to the vessel wall. Thus, when needed, myeloid cells can be enlisted to "fill the blanks" by promoting vascular expansion through a plug-and-go hybrid mechanism. The resulting vascular bed is one in which endothelial cells and myeloid cells coexist to form a contiguous inner lining.

The findings helped resolve much of the controversy around the incorporation of myeloid cells into the endothelium. Previous work relied on bone marrow transplantation following irradiation, particularly because this approach enabled the tracing of newly transplanted bone marrow cells. A significant caveat of this approach, however, was the unintended damage to the endothelium. This damage prevented the emergence of the primary mechanism associated with vascular repair: endothelial cell proliferation. The trick used by Singhal et al. (2018) was to place a radioprotective shield over the upper abdomen while irradiating the animals. This step blocked irradiation from accessing the liver and enabled the authors to clarify the relative contribution of endothelium versus myeloid cells in the process of endothelial cell regeneration and repair. In addition, the investigators subjected animals to alternative modes of chronic liver damage, including administration of carbon tetrachloride and adenoviral infection. In both cases, the endothelium was self-sufficient in its ability to expand. Combined, the multiple approaches uncovered the remarkable self-reliance of the vascular tree,

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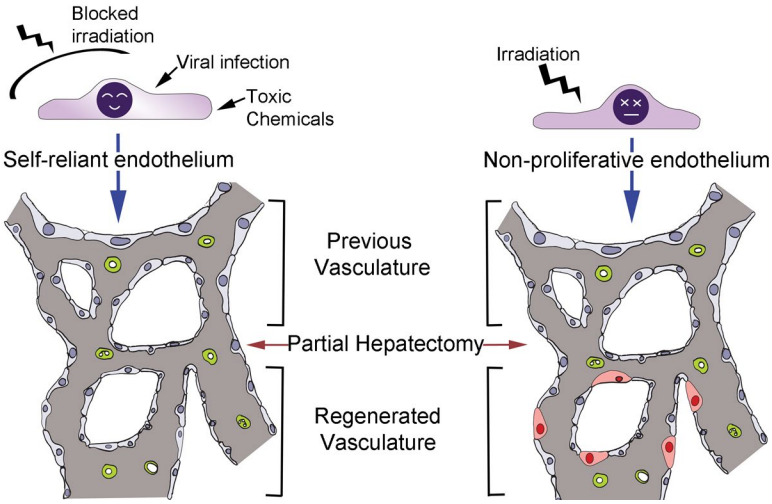

Cellular mechanisms associated with vascular repair. When damage or tissue trauma leaves the proliferative capacity of the remaining endothelium intact (left), repair and expansion of the neovasculature occur through endothelial cell proliferation. If the insult blocks the proliferative capacity of the remaining endothelium (right), circulating bone marrow–derived myeloid cells are recruited to the tunica intima and contribute to vascular repair.

and also facilitated understanding of the interactions with circulating myeloid cells. The bottom line is that, under nonvascular damaging conditions, bone marrow–derived cells do not physically incorporate into the regenerating liver vasculature after partial hepatectomy. However, if the health of the endothelium is significantly impaired to the point of hindering endothelial proliferation, a small proportion of myeloid cells contributes to vascular repair by directly incorporating into the vascular wall.

Because recent studies demonstrated that autologous stem cell grafts resulted in improvement in liver function and regeneration, Singhal et al. (2018) explored whether increasing the mobilization of bone marrow cells could aid or accelerate vascular repair and expansion following partial hepatectomy. The approach followed was to either deliver drugs to mobilize endogenous bone marrow cells or to inject labeled bone marrow progenitors. Neither case resulted in incorporation of bone marrow cells into the vasculature, revealing that increasing the availability of a putative progenitor did not change the outcome.

The resilience of the endothelium after trauma and its endogenous capacity to regenerate have been recently demonstrated in the liver (Wakabayashi et al., 2018) and in the aorta (McDonald et al., 2018). Both studies used genetic tracing and, in one case, parabiosis, to explore the potential contribution of circulating endothelial cells in vascular regeneration. Their findings nicely align with work by Singhal et al. (2018) supporting the notion that, after trauma or partial hepatectomy, endothelial cells are able to proliferate and repair the inner lining of blood vessels, including large vessels like the aorta. That being said, those studies did not explore the process of repair when the proliferative capacity of endothelial cells has been compromised. Another recent set of studies has evaluated the contribution of bone marrow cells to aid in situations of endothelial damage in the central nervous system (Dietrich et al., 2018) and in the bone marrow stroma (Abbuehl et al., 2017). In both cases, the irreparable damage was irradiation.

Naturally, this work raises multiple questions. What stressors or damage would similarly suppress endothelial-mediated repair? And in the case of irreparable damage, what is the impact of the myeloid recruitment? The relative contribution of myeloid cells in situations of endothelial damage was partially explored by Singhal et al. (2018). They found that upon transfer of 5,000 $Lin^{-}Sca^{-}1^{+}Kit^{+}$ (LSK) cells shortly after irradiation, the incorporation of myeloid cells in the entire liver was close to 4%. Unfortunately, there are inherent limitations with the experiment, as the degree of bone marrow engraftment, the time kinetics of endothelial damage preceded LSK delivery, and the proportion of circulating cells needed after hepatectomy were all unclear. Furthermore, it is unknown how many endothelial cells are actually damaged and what the level is of vascular regeneration. Additional exploration to determine the percentage of myeloid cell incorporation in relation to endothelial cell loss will be critical to ascertain their pathophysiological impact. In addition, which myeloid cell is responsible for the repair? The identification of this surrogate population could bring immense clinical benefit. Moreover, are myeloid cells capable of undergoing full endothelial cell differentiation? Can they acquire the same transcriptional fingerprint as liver endothelium? In their study, Singhal et al. (2018) performed microarray analysis comparing the bone marrow cells' recruited cells to the resident liver endothelium. They report that bone marrow cells showed an impressive similarity to the endothelium based on a few markers, but also retained stem cell lineage markers revealing their origin. Clearly, single-cell sequencing analysis could significantly expand the transcriptional profile, bringing clarity to the identity of each cell subtype. This technology could clarify the potential path of differentiation toward an endothelial signature. Finally, is a vascular tree repaired by myeloid cells physiologically similar to another vascular bed repaired through endothelial cell proliferation? Answering this last question would carry relevance to the potential use of these cells in situations of vascular repair. Interestingly, deletion of Notch1 in the adult endothelium impacts junctional complexes and promotes loss of endothelial cells. While endothelial cells are able to proliferate, their impairment in junctional complexes leads to detachment. This is associated with the concurrent seeding of inflammatory/myeloid cells, which fill gaps side-by-side with endothelial cells (Mack et al., 2017). These findings bear some resemblance to the work by Singhal et al. (2018). In the case of the Mack study, however, it was the deficiencies in junctional complexes, not their proliferation ability, that made the tunica intima unable to maintain continuity, a fact that was resolved by myeloid cells. The pathophysiological consequences in this case were severe, as when in the presence of hypercholesterolemia, mice that lacked Notch1 showed a greater percentage of atherosclerosis plaques.

Going back to the initial point of contention, how much of a resolution on the progenitor question has been gained? By

performing both the irradiated and non-irradiated assays concurrently, Singhal et al. (2018) have bridged several gaps. The self-reliance of endothelial cells as main mediators of repair was tested, sufficiently explored, and recognized. The experiments also proved that, if needed, bone marrow cells can be effectively recruited to fill gaps side-by-side with endothelial cells in the tunica intima. Collectively, the findings by Singhal et al. (2018) brought perspective on a large volume of published studies and refined our understanding of how the resilience of the vasculature emerges from multiple mechanisms of expansion ranging from self-reliance to hematopoietic cooperation depending on the circumstances.

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