Aortic Tissue as a Niche for Hematopoiesis

Running title: Yoder; Aortic tissue as a niche for hematopoiesis

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The stem cell theory of hematopoiesis predicts that all mature blood cell lineages circulating in the bloodstream or resident in tissues, arise via a hierarchy of hematopoietic progenitor cell (HPC) intermediates that are ultimately derived from hematopoietic stem cells (HSC). In the adult human subject, bone marrow resident HSC and HPC generate billions of circulating blood cells daily, ranging from the short-lived neutrophils and platelets to long-lived B and T lymphocytes and monocyte-macrophages. Recent interest in the roles of macrophages as modulators of angiogenesis\(^1\) and as participants in atheroma formation\(^2\) highlight the need to better understand the mechanisms of recruitment and retention of macrophages and their progenitor precursors within tissues. In this issue of *Circulation*, Psaltis and co workers\(^3\) identify clonogenic HPC activity in aortic adventitia (that is enriched in HPC compared to peripheral blood or muscle tissue) which appears skewed toward colony forming unit-macrophage (CFU-M) differentiation. In addition, stem cell antigen-1 (Sca-1) expressing cells in the aortic adventitia co-expressed a variety of HSC antigens and adoptive transfer of these cells into irradiated recipient mice led to low but sustained multilineage reconstitution; a property of HSC.

While bone marrow (BM) derived donor hematopoietic cells reconstituted the aorta of lethally irradiated animals, the majority of aorta-derived CFU-M appeared to be constitutive or long term residents of the vessel wall (with sustained but only partial replacement from donor BM cells) suggesting that either the aortic adventitial microenvironment imparts specific differentiation properties to marrow-derived precursors or that a local origin for HSC/HPC exists in the adult vasculature. Is there any evidence to suggest that HSC/HPC may originate and/or migrate through the aortic adventitia?
Hematopoiesis occurs in a site and tissue specific pattern that varies throughout mammalian development. The first blood cells (including macrophages) arise in the extra-embryonic yolk sac and the yolk sac is the principle source of all the erythro-myeloid progenitor cells that migrate through the blood stream to seed the fetal liver at the 30 somite pair stage of development (embryonic day 10 [E10]) \(^4\). The first HSC (that upon transplantation repopulate hematopoiesis in lethally irradiated adult mice) emerge from hemogenic endothelial cells on E10.5 from the ventral intimal wall of the dorsal aorta (in an area identified as the aorta-gonad-mesonephros or AGM region) and are followed on E11 by HSC arising from hemogenic endothelial cells lining the vasculature of the yolk sac, placenta, and the fetal liver (in addition to the vitelline and umbilical arteries)(reviewed in\(^5\)). By E14, the fetal liver is the predominant site for HSC and HPC residence and all other hematopoietic sites decline in blood forming activity. HSC from the fetal liver subsequently migrate via the circulation to seed the bone marrow compartment prior to birth and the bone marrow becomes the predominant site for hematopoiesis throughout adult life. Thus, the predominant sites of hematopoiesis change throughout mammalian development and the circulation appears to be a major pathway through which HSC/HPC migrate from one tissue to another.

Of relevance to the present work of Psaltis and coworkers\(^3\), some controversy continues to exist with respect to the origin of the first murine embryonic HSC emerging from the ventral wall of the dorsal aorta. A number of investigators have provided convincing cell-fate tracing evidence that hemogenic endothelial cells of the dorsal aorta directly give rise to the HSC that comprise the fetal liver HSC pool and ultimately, constitute the medullary HSC pool in the adult mouse (reviewed in\(^5\)). However, other investigators have provided evidence that the subaortic
mesenchyme may serve as a source of HSC\(^6\) (or pre-HSC\(^7\)) that may migrate through the endothelial cells to gain access to the circulation or may transition into a hemogenic endothelial intermediate prior to budding into the aortic lumen. Since this developmental question remains unresolved, one must wonder if the mesenchymal origin of the pre-HSC identified in the embryonic AGM in any way may contribute to the presence of the HSC/HPC identified in the present study of Psaltis et al.\(^3\) in the adult aortic adventitia. However, if HSC do not originate within the aortic adventitia of the embryonic aorta and persist in the adult subject, how would HSC/HPC activity become enriched in this tissue in the adult mouse?

It is well known that HSC, as well as HPC, constitutively circulate throughout the bloodstream of adult mice\(^8\). However, it has only recently become evident that circulating HSC emigrate into tissues, reside for several days, then exit via the lymphatics to regain entry into the bloodstream; indeed several hundred HSC and HPC migrate throughout the lymph fluid daily in the adult mouse\(^9\). Recovery of the HSC from the lymph fluid and transplantation into lethally irradiated host animals has provided evidence that the donor cells are capable of multilineage repopulation in primary and secondary recipient mice; defining the cells as *bone fide* long-term repopulating HSC. Of interest, similar HSC activity was obtained from murine lung, liver, spleen, and kidney tissue demonstrating that HSC migration occurs in non-lymphoid, as well as, lymphoid tissues with a dwell time in each site of approximately 48h. Of interest, recruited donor HSC/HPC were noted to proliferate and give rise to primarily myeloid cells at steady state conditions and these activities were enhanced when the host was challenged by lipopolysaccharide (LPS) injections into various tissues. In fact, LPS injections into organs not only promoted HSC/HPC proliferation and differentiation, but prevented the exit of clonogenic myeloid cells from these
tissues. These data have suggested that migratory HSC/HPC may contribute to resident tissue myeloid pools, particularly during states of inflammatory stress.

Psaltis et al.\(^3\) reported that the CFU-M identified in aortic tissue was found at near similar frequencies in other large vascular tissues (carotid and femoral but not renal arteries). While the authors did not pursue further studies on the mechanisms that cause the renal arteries to be deficient in retention of hematopoietic colonies, these data are consistent with some prior published work. While mesenchymal cells derived from a variety of vessels and tissues are largely supportive of hematopoiesis\(^10\), Li et al.\(^{11, 12}\) have reported that primary endothelial cells isolated from various vascular beds in embryonic and adult mice differ in their in vitro support of HSC and HPC. Murine yolk sac- and intraembryonic para-aortic splanchnopleure-derived endothelial cells promoted significant expansion of adult BM-derived HSC and HPC during 4-7 days of in vitro co-culture\(^{11}\). However, isolation of primary endothelial cells from adult murine organs and co-culture with BM-derived hematopoietic cells revealed that brain and heart endothelium significantly increased, lung and liver endothelium maintained, and kidney endothelial cells markedly decreased the number of CFU during a 7 day co-culture compared to the freshly isolated BM-derived hematopoietic cells\(^{12}\). HSC competitive repopulating activity was maintained in heart and liver endothelial co-cultures with BM-derived hematopoietic cells while all HSC activity was lost in BM-derived hematopoietic cells co-cultured with primary kidney endothelium even when co-cultured in the presence of known hematopoietic growth factors that support HSC and HPC maintenance and differentiation in vitro\(^{12}\). These studies have suggested that kidney endothelial cells are detrimental to HSC and HPC survival in vitro and with the new information supplied by Psaltis et al.\(^3\) suggest that a similar inhibitory
microenvironment for hematopoietic cells may exist in the renal arteries. Identification of the mechanisms that cause the renal vascular bed to lack hematopoietic supportive properties displayed by other arteries may be of great interest given the known homeostatic requirement for erythropoietin production by specific renal cortical cells.\textsuperscript{13}

Psaltis et al.\textsuperscript{3} also reported that CFU-M frequencies were significantly higher in aortic tissue samples isolated from pro-atherogenic apolipoprotein E-null (\textit{ApoE}\textsuperscript{-/-}) mice even though the aortic samples were atheroma lesion free at the time of tissue harvest (to avoid detection of myeloid precursors derived from inflammatory atheroma). Expression of Sea-1 by HPC was increased in the \textit{ApoE}\textsuperscript{-/-} aortic tissue and essentially all clonogenic CFU activity was retained by this cell subset. Of interest, after bone marrow transplantation, the majority of Sea-1\textsuperscript{+} myeloid progenitor cells retained in the aortic tissue in both wild-type and \textit{ApoE}\textsuperscript{-/-} mice were of host rather than donor origin (in contrast to near complete bone marrow reconstitution by the donor cells). These data suggest that the aortic adventitia may provide unique signals to promote skewing of migrating HSC into CFU-M or permit the preferential long-term residence of a CFU-M population. While there is evidence that some mature macrophage populations in various tissues display proliferative potential to replenish resident macrophage pools\textsuperscript{14-16}, there is no compelling evidence that a macrophage progenitor population can self-renew long term in any tissue; essentially all progenitor pools must be replenished by HSC input. If CFU-M are not renewed in situ, how might the aortic adventitial microenvironment lead to a skewing of recruited HSC into CFU-M?
HSC display heterogenous behaviors with respect to the composition, kinetics, and persistence of progeny lineage outputs when following the repopulation patterns of donor HSC at a clonal level. Indeed, Dykstra et al.\textsuperscript{17} have reported that one pattern of clonal HSC repopulation results in primarily a myeloid predominant reconstitution (limited lymphoid repopulation) that is persistent through secondary and even tertiary transplants. This kind of behavior has also been reported by others and appears to become more prevalent with advancing age in mice\textsuperscript{18, 19}. Given the high CFU-M content of the aortic adventitia reported by Psaltis et al.\textsuperscript{3}, one wonders if this particular type of myeloid repopulating HSC is preferentially recruited into the macrovascular adventitia and retained to a greater extent within the tissue than other migrating HSC with more balanced or lymphoid predominant patterns of reconstitution. Alternatively, perhaps the factors which contribute to the distinct features of the aortic adventitia (cellular composition, extracellular matrix proteins and associated specific biomechanical properties, oxygen tension, and growth factor milieu) may stimulate a predilection for skewed differentiation of HSC into a particular lineage. As noted above, binding of LPS via HSC toll-like receptors (TLR) and associated binding proteins can enhance preferential differentiation of HSC into myeloid progenitor cells and enhance their retention within the sites of TLR activation. The role of toll-like ligands and cells expressing the TLRs in the pathophysiology of atherosclerosis\textsuperscript{20} provide an intriguing pathway for future studies that may discover a role for the aortic adventitial myeloid cells in disease initiation, progression, and if inhibited perhaps amelioration or even prevention.
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