Abstract—A classic study in 1968 proposed that bone marrow-dwelling promonocytes differentiate to monocytes, which then intravasate, circulate, and, on tissue entry, differentiate to sessile macrophages. Since then, understanding of the macrophage family relationship has undergone substantial enhancement and occasional revision. It is currently recognized that in addition to their role in the bone marrow, hematopoietic progenitors circulate and give rise to their descendants in extramedullary niches. Monocytes, of which there are several subsets, are not merely circulating macrophage precursors but participate in the immune response in their own right. Macrophages are highly heterogeneous and, as recent studies indicate, can arise in the absence of a monocyte intermediate. These spatial and developmental relationships reveal a complex interactive network and underscore the importance of context in evaluating biological systems. The observations have significant implications for how we image, target, and treat disease. (Arterioscler Thromb Vasc Biol. 2011;31:1517-1522.)

Key Words: blood cells ■ immune system ■ leukocytes ■ macrophages ■ hematopoiesis

The purpose of Ralph van Furth and Zanvil A. Cohn’s experiments was to understand the in vivo relationship between “free and fixed mononuclear phagocytes in different parts of the body under normal, steady state conditions.”1 It was already known that monocytes and macrophages exist and that they participate in clearing foreign and infectious agents. It was also known that monocytes give rise to macrophages in inflammation. The in vivo origin and turnover of monocytes in the steady state, however, was not yet understood. Through a series of labeling and fate mapping studies, the authors concluded that rapidly dividing bone marrow promonocytes give rise to circulating monocytes, which differentiate to tissue macrophages.

This linear and spatially constrained framework has influenced our thinking on how macrophage-lineage cells relate to each other. Numerous studies have shown that the bone marrow produces hematopoietic cells, and much research is currently devoted to understanding the cellular and molecular components that constitute the stem cell bone marrow niche. Monocytes and macrophages have, likewise, received considerable attention, which has yielded a rich and nuanced understanding of their participation in health and disease. It may therefore be instructive to revisit the 2 pivotal components of van Furth and Cohn’s model. The first is topographical: monocytes circulate in the blood, whereas progenitors and progeny reside in tissue. The second is developmental: bone marrow progenitors give rise to macrophages through a monocyte intermediate. In this review, I will discuss a few aspects of progenitor, monocyte, and macrophage biology, illustrated in the Figure, focusing particularly on recent studies that have challenged these 2 assertions.

Hematopoietic Progenitors: Protected Dwellers or Roving Travelers?

Hematopoiesis occurs in the bone marrow of the healthy human or mouse. The bone marrow stem cell niche is a highly organized environment consisting of supporting cells and molecules that guide stem cells through phases of self-renewal, proliferation, differentiation, and mobilization.2–6 Its various cellular components include mesenchymal stem cells, osteoblasts, adipocytes, endothelial cells, and macrophages. Osteoblasts, which derive from mesenchymal stem cells, produce hematopoietic cytokines and mediators that influence stem cell expansion, repopulating capacity, and retention.7–9 Adipocytes, which are also of mesenchymal origin, regulate the bone-marrow environment, possibly through adiponectin,10,11 whereas CD169+ macrophages promote the retention of hematopoietic stem cells (HSC) in the bone marrow niche.12 Such accessory cells secrete cytokines, growth factors, chemokines, and other molecular mediators and, together with intrinsic stem cell factors,13–16 contribute to the bone marrow’s role as a housekeeping site of leukocyte production.

Blood transplantation experiments in mice provided the first evidence, in 1962, that HSC circulate.17 It was later shown that HSC circulate freely and can reengraft vacant...
bone marrow niches. The mechanism behind HSC mobilization is not yet clear but is known to involve granulocyte colony stimulating factor, a growth factor long recognized in the clinic for its mobilizing activity. Granulocyte colony stimulating factor activates neutrophils, which then secrete neutrophil elastase and cathepsin G. The proteases function in the bone marrow in at least 2 ways: first, they sever interactions between stem and stromal cells by cleaving VCAM-1; second, they interfere with the stromal-derived factor–CXCR12 chemokine axis that otherwise keeps HSC in the bone marrow niche. More recent work has shown that the hormone noradrenaline contributes to HSC mobilization by decreasing CXCL12 transcription. This sympathetic nervous system hormone’s intriguing role provides a mechanism for the robust circadian fluctuations of circulating HSCs and indicates that mobilization is a well-controlled process.

Several non–mutually exclusive theories about the physiological role of HSC mobilization have been proposed. According to 1 theory, HSC mobilization reflects bone marrow niche reconstruction. This idea is based on the observation that the increase in HSC numbers in mouse peripheral blood during daylight correlates with higher bone-remodeling activity. Given the interdependence of bone and bone marrow, it is niche reconstruction that “sends HSCs out of their home.” During periods of darkness, when mice are most active and bone remodeling is lowest, HSC return to the bone marrow and compete for space in the vacant niches. The system thus selects for the most fit cells capable of reengraftment.

HSC may also mobilize for tissue immunosurveillance. In support of this concept, hematopoietic stem and progenitor cells (HSPC) have been shown to migrate to multiple peripheral organs and either differentiate to tissue resident cells or recirculate through the lymphatics back to the bone marrow. HSPC egress from such extramedullary sites depends on sphingosine-1-phosphate and is perturbed during inflammation. To evade phagocytosis in the harsh extramedullary environment, HSPC upregulate CD47, a cell surface “don’t eat me” marker that interacts with receptor signal regulatory protein-α on scavenging macrophages. Together, these observations lend further support to the theory that HSC mobilization has an important physiological function beyond bone marrow niche reconstruction.

Extramedullary hematopoiesis is a well-documented phenomenon that occurs in both humans and rodents. In addition to supporting hematopoesis during development, extramedullary sites in the adult can, under certain conditions, generate platelets, erythrocytes, granulocytes, and mononuclear phagocytes. There is an obvious advantage to outsourcing the production of terminally differentiated leukocytes to peripheral organs: HSPC that establish residence in a target organ can proliferate, generating many descendants. The process is self-sustaining and less dependent on constant replenishment of bone marrow–derived nonproliferating cells, but it can also backfire, as documented in various genetic and myeloproliferative diseases. Despite the many situations where extramedullary hematopoiesis occurs,
its orchestrating mechanisms and the scope of its influence remain largely unexplored. One study, aiming to decipher what an extramedullary niche may look like, proposed that, in the absence of osteoblasts, HSC settle adjacent to the sinusoidal endothelium. HSPC retention and survival may depend on sphingosine-1-phosphate and CD47, respectively, but other supporting cells and molecules remain undescribed. It is also unclear whether extramedullary hematopoiesis can give rise to circulating monocytes that populate tertiary sites or whether the process necessarily differentiates cells to their terminal tissue phenotype. Future studies will need to investigate the functional and compositional properties that characterize the extramedullary niche, compare progeny made in different locations, and investigate whether such processes participate in various acute and chronic diseases.

**Monocytes: Indifferent Progenitors or Resolute Gatekeepers?**

Monocytes are defined as large bone marrow–derived circulating mononuclear phagocytes that give rise to macrophages and dendritic cells. The cells arise in the bone marrow from a dedicated clonogenic progenitor and enter the circulation via chemokine (C-C motif) receptor 2. In the blood, monocytes circulate freely for a few days before extravasating or dying, and in vitro, they rapidly differentiate. Monocytes are thus transitional cells that bridge the gap between clonogenic progenitors and terminally differentiated macrophages.

Leukocyte intravasation is well described and includes such processes as capture, rolling, arrest, adhesion, crawling, and transendothelial migration. To enter tissue, leukocytes cross several layers: they pass through a confluent layer of endothelial cells and a nonconfluent layer of pericytes. Penetration of the endothelium occurs either by transcellular migration (through cells) or by paracellular migration (between cells), although the relative importance of transcellular migration is not known. The entire process involves a myriad of adhesion molecules and various proinflammatory and hydrostatic forces.

Leukocyte engagement with the endothelium does not necessarily lead to transmigration. Mouse and human monocytes, for example, patrol the endothelium in the steady state. The process depends on lymphocyte function–associated antigen-1, does not follow the direction of blood flow, precedes extravasation, and is limited to Ly-6C(low) monocytes in the mouse and CD14(low) monocytes in the human. (See indicated references on monocyte heterogeneity.) The patrolling supports the idea that monocytes are sentinels that screen for injury or infection. It also indicates that monocytes are active gatekeepers of the inflammatory cascade. Neutrophils, the other myeloid circulating cells, have also been shown to crawl along the endothelium, but it is not yet clear how the 2 processes compare.

It is generally accepted that once a monocyte transmigrates, it differentiates. It has therefore been surprising to discover that monocytes can also inhabit tissue without differentiating. Clusters of monocytes that are indistinguishable from their circulating counterparts inhabit the splenic red pulp. The splenic monocytes are relatively immotile in the steady state, but in response to distant injury they can invasate and populate distant tissues. This monocyte reservoir not only contributes to injury associated with myocardial infarction and abdominal aortic aneurysm but has also been implicated in ovulation, stroke, and several other conditions (unpublished observations). It is still not known how this reservoir is replenished and maintained, and it is possible that the spleen, perhaps by virtue of its open circulation, is uniquely suited to accommodate vast quantities of undifferentiated monocytes. Nevertheless, it is appealing to speculate that monocytes can enter and exit tissue without differentiating because it indicates that monocytes, as monocytes, influence the immune response outside of the blood.

**Macrophages: Ontogenically Uniform or Altogether Heterogeneous?**

Given monocytes’ image as progenitors, it is not surprising that their fate has received considerable attention. The list of tissues that monocytes are known to infiltrate is long. In the inflamed skin, Ly-6C(high) monocytes differentiate to Langerhans cells. The inflamed brain sees Ly-6C(high) monocytes accumulate and give rise to microglia. A model of myocardial infarction reveals Ly-6C(high) and Ly-6C(low) monocyte accumulation occurring in 2 phases. First, inflammatory Ly-6C(high) monocytes accumulate via chemokine (C-C motif) receptor 2, phagocytose dead cells, and secrete proteases. Then, 4 days later, reparative Ly-6C(low) monocytes accumulate via CX3CR1 and contribute to granulation tissue formation. In a nonischemic model of skin injury, Ly-6C(high) monocytes accumulate and polarize to different macrophage phenotypes over time. A model of heart transplantation shows Ly-6C(high) monocytes progressively infiltrating the allograft and contributing to a signature of organ rejection, but the monocytes can also induce tolerance. Studies of atherosclerosis indicate that Ly-6C(high) monocytes give rise to lesional macrophages, whereas Ly-6C(low) monocytes appear to differentiate to dendritic cells. In adipose tissue, M1 macrophages appear to be of Ly-6C(high) origin. Bacterial infection induces Ly-6C(high) monocytes to generate tumor necrosis factor–α and inducible nitric oxide synthase, thereby becoming so-called tumor necrosis factor–α/inducible nitric oxide synthase–producing dendritic cells (TipDC). Lastly, there is now convincing evidence that monocytes can acquire dendritic cell morphology, localize in T-cell areas, and stimulate adaptive immunity by presenting and cross-presenting antigen. (See indicated references for a discussion of how macrophages and dendritic cells compare.)

An enormous body of literature confirms that macrophages and dendritic cells have a role in diseases such as atherosclerosis, obesity, and cancer, and many of these cells are known to derive from monocytes. Whether monocytes or their subsets differentiate to specific macrophage or dendritic cell populations in the steady state is less clear. Recent work has shown that microglial cells arise from primitive Runx1+ resident progenitors and not from monocytes. This surprising disconnect can also be observed between monocytes and dendritic cells. Even though dendritic cells can arise from monocytes during inflammation,
classical splenic dendritic cells and, presumably, Langerhans cells arise without a monocyte intermediate in the steady state. Collectively, these studies suggest that monocytes are not, by default, tissue-replenishing cells.

What then is monocytes’ physiological fate? Data on this are scarce because it is difficult to conduct convincing monocyte tracking experiments, and those that exist tend to focus on disease models. Perhaps, as van Furth and Cohn proposed, monocytes repopulate the peritoneal macrophage pool. This is not a very satisfying explanation when cell number and turnover kinetics are considered: if monocytes do indeed circulate briefly, then their turnover in the peritoneum should be very rapid, and this is unlikely given the manifest morphological and phenotypic differences between the cells. It is more satisfying to think that, in the absence of differentiation-inducing signals, monocytes circulate, patrol, and accumulate in reservoir sites. Unless they encounter a differentiation signal, they are simply eliminated through efferocytosis. If so, it may be that such efferocytosis-mediating machinery also regulates monocyte production. It is also remotely possible that monocytes give rise to other, unexpected phenotypes, although, again, the rate of monocyte turnover suggests such contribution to be minor. Clearly, there is a need for a systems-wide approach to dissect the monocyte life cycle in its various spatiofunctional contexts.

Concluding Remarks

Van Furth and Cohn’s influential 1968 study has shaped our understanding of the spatial and developmental relationships between monocyte precursors, monocytes, and their progeny. Accumulating evidence indicates a more complex series of interactions than previously believed: progenitors circulate and differentiate in extramedullary niches, monocytes are not simply transitional, and macrophages can develop without a monocyte intermediate. This complexity, which requires further attention to context, enriches rather than invalidates Van Furth and Cohn’s observations. Such complexity has implications for how we treat disease because understanding of the spatial and developmental relationships between cells and for how long during treatment. Approaches that target the macrophage lineage either for imaging or treatment will require a nuanced understanding of the cells’ distribution, function, and life span (on molecular imaging of macrophages, see Leuschner and Nahrendorf). The insights may lead to tailored targeting strategies that discriminate between beneficial and harmful functions for which macrophages and their associated cells are known.

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