Neutrophils Usher Monocytes Into Sites of Inflammation

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One of the key processes of inflammation is the transmigration of circulating leukocytes across the endothelium. Among the leukocytes, neutrophils and monocytes are large phagocytes that can respond quickly to infection or injury. On sensing danger, neutrophils and monocytes adhere to the endothelium and transmigrate to the adjacent tissue via the coordinated activities of adhesion molecules, integrins, cytokines, and chemokines. Once they accumulate, these myeloid cells participate in a myriad of immune inflammatory activities. The importance of this event cannot be understated, especially because the accumulation of leukocytes in tissue is a double-edged sword. On the one hand, coordinated leukocyte accumulation in injured or infected sites is required for effective pathogen elimination and tissue healing. On the other hand, uncontrolled accumulation is a defining feature of chronic diseases, such as atherosclerosis. Understanding leukocyte migration is essential to understanding the immune system.

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Neutrophils and monocytes do not accumulate all at once. In a typical acute inflammatory response, there is a well-defined sequence: neutrophils accumulate first; monocytes accumulate second. Among the monocytes, of which ≥2 subsets circulate in the mouse and human, there is yet another sequence: inflammatory murine Ly-6C<sup>high</sup> monocytes accumulate first and reparative Ly-6C<sup>low</sup> monocytes accumulate second. This temporal (neutrophil → Ly-6C<sup>high</sup> monocyte → Ly-6C<sup>low</sup> monocyte) hierarchy of accumulation is likely required for an effective innate response. The subsets, which have overlapping but also specialized functions, contribute sequentially to processes that involve pathogen elimination, efferocytosis, restoration of tissue integrity, and the initiation of adaptive immunity. How are these accumulation sequences orchestrated? Although the molecules that guide leukocyte arrest, firm adhesion, and transmigration can explain the process in mechanistic detail, they do not reveal the larger context of influence among leukocyte classes.

Platelets have long been recognized for their ability to help monocytes accumulate in tissue. It is therefore not surprising that other cells (or, in the case of platelets, cell fragments) can act as chaperones for monocytes. Neutrophils are excellent chaperone candidates because they are among the first to respond and because they contain large quantities of preformed granular proteins. In this issue of *Circulation Research*, Wantha and colleagues provide mechanistic detail as to how extravasated neutrophils may be alerting monocytes.

In their previous work, the authors have demonstrated that neutrophil-derived cathelicidin promotes endothelial adhesion of classical monocytes to the large arteries during experimental atherosclerosis. Here, Wantha et al elucidate specific mechanisms that control this process (Figure). Using an in vivo model of tumor necrosis factor-α–mediated endothelial activation of the cremaster muscle, the authors show that endothelial adhesion of Ly-6C<sup>high</sup>, but not Ly-6C<sup>low</sup>, monocytes is attenuated in cathelicidin-deficient mice (Cramp<sup>−/−</sup> mice). CRAMP, they show, is neutrophil derived and accumulates on the luminal surface of the endothelium in a proteoglycan- and caveolin-independent manner. Importantly, in vitro studies demonstrate that human cathelicidin (LL37) similarly enhances adhesion of classical (CD14<sup>+</sup>CD16<sup>−</sup>), but not nonclassical (CD14<sup>+</sup>CD16<sup>+</sup>), monocytes. Cathelicidin interacts with formyl-peptide receptor 2, which is preferentially expressed on classical monocytes, but not formyl-peptide receptor 1 or other cathelicidin receptors, including CXCR2 and P2X7. Cathelicidin binding of formyl-peptide receptor 2 induces activation and conformational changes of the integrins Mac1 (CD11b/CD18) and VLA-4 (CD49d), increasing binding of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, respectively. Mutational analysis of LL37 revealed that cell adhesion and integrin activation depend on the central FKRIV motif.

In addition to its antimicrobial properties, cathelicidin has known immunomodulatory functions. In this study, Wantha et al identify a novel function of cathelicidin in regulating inflammation: the adhesion of classical monocytes to the inflamed endothelium. Although integrin-mediated endothelial attachment is critical for the recruitment of leukocytes to inflamed sites, the extent to which cathelicidin controls monocyte accumulation in the tissue remains to be determined. In addition, it will be necessary to establish whether cathelicidin mediates adhesion beyond tumor necrosis factor-α stimulation and at tissue sites other than the cremaster muscle. In other words, is cathelicidin-mediated monocyte adhesion a general feature of inflammation? The previous work of the authors in atherosclerosis suggests this is likely. Finally, there may be therapeutic implications to cathelicidin preferentially regulating endothelial adherence of classical monocytes. Targeting cathelicidin, formyl-peptide receptor 2, or their interaction may dampen...
excessive inflammation without disturbing processes typically attributed to nonclassical monocytes, including wound healing, tissue remodeling, and resolution of the inflammatory response.

The immune system is characterized by leukocyte communication. The generation of immune memory, for example, relies on cross-talk between antigen-presenting dendritic cells and antigen-responsive lymphocytes. Antibody-producing B lymphocytes rely on help from T cells for isotype switching, and interferon-γ-producing lymphocytes arm macrophages for efficient bactericidal activity. Leukocyte accumulation in tissue, it turns out, is also heavily influenced by leukocyte communication. The study by Wantha et al. challenges us to think about leukocyte flux not just in terms of migration of discrete leukocyte subsets but also in the larger interdependent context of cell and organ systems.

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References

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