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Leukocyte Chemotaxis and Migration

Can We Follow the Cells?

A CRITICAL feature of the innate immune response is the movement of neutrophils and macrophages from one site in the body to another to provide effector functions. These immune cells play a critical role in fighting infection but may also cause damage to parenchymal cells by releasing oxidants and proteases. Although the molecular mechanisms of leukocyte chemotaxis during inflammation have been investigated with great detail,[1] the measurement of the migration of immune cells into organs or body cavities still rely on the lavage of these cavities to obtain immune cells, or on the sampling of tissues for quantification of markers of immune cell activity, such as the myeloperoxidase produced by neutrophils. These tests are invasive and not easily performed in humans; however, recent advances in intravital imaging techniques, together with advances in molecular probes and reporters for cell labeling, have made it possible to visualize these cellular processes in live animals as the immune response unfolds in real time. For example, intravital microscopy techniques have been used to study the interactions between leukocytes and the vascular endothelium.[2] Furthermore, *in vivo* homing assays have been used to assess target recruitment of leukocytes in response to inflammatory stimuli; but these assays require postmortem analyses of leukocyte tissue distribution.[3] In contrast, *in vivo* studies of the migration of leukocytes into inflamed body cavities have been difficult because of the lack of tools to measure leukocyte trafficking. In this issue of the Journal, Dr. Larmann and colleagues propose to use a novel intravital imaging technique to longitudinally quantify cell trafficking in inflammatory models in live animals.[4] The approach used by these investigators relies on the use of *in vivo* near-infrared fluorescence imaging, which is an emerging imaging technique that allows *in vivo* sensitive detection and quantification of fluorescent probes. Fluorophores emitting in this spectrum penetrate tissues efficiently and can be easily detected with a novel three-dimensional fluorescence-mediated tomography.

The study by Larmann *et al.*[4] is important for several reasons. First, using fluorescence-mediated tomography, these investigators were able to follow the migration of leukocytes in two important body cavities: the peritoneum and the distal airspaces. It is noteworthy that the intensity of the inflammatory cell response in their model of thioglycollate peritonitis was largely underestimated by lavaging the peritoneum, a classic technique that is used to evaluate the number of leukocytes that accumulated in that cavity. Second, the investigators demonstrated the feasibility of their proposed technique by reporting that the staining of the immune cells did not affect their ability to adhere to the vascular endothelium, as well as to migrate across this cell barrier. In addition, the viability of the labeled cells remains excellent with only a minor fading of their fluorescence 3 days after injection. Third, this new technique provides novel information about the dynamics of an inflammatory process. Fluorescence-mediated tomography is capable of detecting low numbers of labeled immune cells that are recruited in a body cavity, as well as the trafficking of these cells between different organs. Fourth, this study also suggests that the potential use of optical imaging to follow leukocytes trafficking in humans as fluorescence reflectance imaging techniques can easily be miniaturized. Handheld optical devices that are under development could be used in the future to scan, for example, the abdominal cavity or any other inflammatory site to yield information about leukocyte recruitment and the evolution of any inflammatory process. Finally, this study provides new and important information about the role of the urokinase receptor in modulating the trafficking of immune cells in response to an inflammatory process. This study provides new experimental evidence that the urokinase receptor not only plays an important role in the trafficking of neutrophils, but also is involved in the recruitment of macrophages to the site of an inflammatory process, with this process depending on the urokinase receptor expressed on leukocytes rather than on endothelial cells.

Despite the novelty of the fluorescence-mediated tomography technique to monitor *in vivo* the movement of leukocytes toward the site of an inflammatory process, the study by Larmann *et al.*[4] has several limitations. First, the authors used peritoneal leukocytes instead of peripheral blood immune cells. Although these peritoneal leukocytes have some similarity with regard to their adhesive and migratory function

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compared with peripheral blood leukocytes,[5] future studies should include the use of the peripheral blood leukocytes. Second, apoptotic labeled cells may have been taken by macrophages, and, thus, the DiR-label may have been transferred to cells other than the one injected into the animals. To address this issue, the investigators used a double-labeling strategy (green fluorescent protein and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide) and demonstrated that injected double-labeled cells were found in the different organs studied. Finally, the experimental protocol used by Larmann *et al.*[4] did not examine the migration of neutrophils and monocytes into different organs at the same time. This would be an important future development for the potential clinical use of this novel technique.

In summary, Larmann *et al.*[4] should be congratulated for the development of a new noninvasive experimental approach to investigate longitudinally leukocyte trafficking within one organ and/or between organs. This new technique will provide us with an invaluable tool to study the dynamics of the innate immune response to trauma or infection. This work also extends our knowledge about the function of the urokinase receptor on neutrophils and suggests potential future applications for the use of this approach in critically ill patients.

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