Fluorescent Leukocytes Enter Plaque on the Microscope Stage

Matthias Nahrendorf, Filip K. Swirski

Atherosclerosis is a disease driven by lipid accumulation and leukocyte influx in the vessel wall.1 Because visualizing leukocyte behavior in vivo is the holy grail for those interested in understanding leukocyte migration, major progress has been made in developing intravital microscopy approaches to study leukocyte movement. Understanding how certain leukocytes, such as T cells, participate in various diseases including cancer2,3 has been one of intravital microscopy’s success stories. The technology has also proven beneficial for understanding basic processes of leukocyte biology, such as the patrolling behavior of monocytes,4 monocyte movement under peristaltic, and cardiovascular motion. The beating heart (and to a somewhat lesser degree, the arterial vasculature) in a live animal moves rapidly with a high amplitude of motion. Without sophisticated stabilization methods, cardiovascular motion makes it impossible to focus on a cell residing in tissue, let alone isolation and resolution of the cell’s movement or subcellular structures. This is one reason why the majority of intravital microscopy studies have focused on locations far from the influence of the heart, such as surgically exposed mesenteric vein,5 lymph node,6 or spleen.7 In cancer imaging, window chambers have allowed for complete tissue immobilization.8 Previous efforts aiming at imaging leukocytes in the vasculature required exposure of the aorta9 or its complete removal from the animal.10

During the past several years, there has been real progress at imaging those most difficult sites close to, or even within, the beating heart. Several studies have reported microscopic fluorescence imaging of the beating mouse heart recently.12,13 In these studies, several innovations served to reduce motion artifacts. First, cardiac tissue was stabilized. One specific solution involved immobilization of the myocardial tissue in the field of view with suction.13 Here, the objective of microscope was placed inside a larger tube to which negative pressure was applied, thus gently approximating and immobilizing the epicardial surface on the objective. An alternative solution used a custom-built tissue immobilizer.12 This ring, which encircled the field of view, was attached to the epicardial surface with tissue glue. The approach was combined with synchronized mechanical ventilation to circumvent respiratory motion and with the use of an ECG for triggering acquisition and image reconstruction.12

In the current issue of Circulation Research, Chevre et al14 describe a method for 2 photon microscopy of atherosclerotic inflammation in the carotid artery in live mice. To follow intraluminal events, the authors stabilized the carotid arteries of Apoe–/– mice at different disease severities. The vessel was surgically exposed, and a metal stage was inserted beneath. A cover slip was then placed on the artery and attached to the stage with clay, leaving ≈400 μm of space for the imaged artery, and thus reducing the pulsatile motion caused by the arterial blood flow. Validation experiments showed that trapping the artery between the metal stage and the translucent cover slip drastically reduced motion in the z direction without compromising the blood flow in the arterial lumen.

The authors then used this technique for imaging luminal behavior of leukocytes such as crawling and rolling, demonstrating slower leukocyte velocity with progressing disease (Figure). Of note, only 2.1% of rolling leukocytes firmly arrested for recruitment, raising the notion that leukocytes readily disengage from plaque endothelium to re-enter circulation, a behavior that is perhaps akin to that of hesitant neutrophils.15,16 Several classes of leukocytes were studied, including neutrophils, monocytes, lymphocytes, and platelets, using a combination of fluorescent reporter genes and fluorescently labeled antibodies that were injected intravenously before imaging. Leukocyte adhesion, and by extension endothelial dysfunction, was spatially heterogeneous. Lesion shoulders were particularly active regions, with platelets homing to myeloid-rich areas. Interestingly, T cells only migrated at later time points (at 6 weeks but not 2 weeks of high-fat diet), whereas myeloid cells always migrated, and neutrophils increasingly so at the later time points. An important methodological difference in the detection of myeloid and lymphoid cells was that the former were identified by green fluorescent protein expression, whereas the latter were labeled with injection of fluorescent antibodies. It will be important to determine whether these 2 approaches result in different sensitivities. Likewise, the differential recruitment between lymphocytes

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and myeloid cells warrants further study, especially because it may reflect processes related to route of recruitment (intimal versus adventitial), antigen recognition (which could explain the later recruitment of lymphocytes), and the generation of adaptive immunity. Indeed, the later appearance of lymphocytes should be reconciled with previous studies showing decreased, rather than increased, density of lymphocytes in lesions of ApoE−/− and LDL−/− mice with disease progression.17

The ability to image behavior of several types of cells allowed Chevre et al14 to test for cell interaction and function in vivo. It is known that monocyte–platelet aggregates promote atherosclerosis.18 The mechanistic details of such aggregates received recent attention,19 and here, Chevre et al14 provide evidence that leukocyte–platelet aggregation involves not only monocytes but also neutrophils. Specifically, the authors describe that platelets rather bind to green fluorescent protein− myeloid cells than to endothelium and that systemic neutrophil depletion with an anti-Ly6G antibody abolishes homing of platelets to the observed vascular structure. On accumulation, monocytes differentiate to macrophages. In this study, Chevre et al14 profile CD62L, a surface marker whose expression decreases with differentiation. The authors show that with increasing adhesion time, CD62L expression on neutrophils decreases, thus opening the exciting prospect of visualizing differentiation and polarization of transmigrating leukocytes in vivo.

The work by Chevre et al14 advances intravital microscopy imaging for the study of leukocyte behavior in atherosclerotic vessels. By using motion compensation strategies in ApoE−/− mice, which express fluorescent reporter proteins in myeloid cells, the authors add to the growing body of work devoted to improving live microscopy of leukocytes in the cardiovascular system. There should be little doubt that applying these high-resolution imaging strategies to research on atherosclerosis will shed new light on role of leukocyte in disease initiation, progression, and complication.

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References


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Figure. The paper by Chevre et al describes a technique for visualizing leukocyte movement to atherosclerotic lesions in live mice.
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