Commentary

A massage for the journey: Keeping leukocytes soft and silent

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You see, the phenomena called forth in vascular parts by the most heterogeneous agencies constitute a perfectly regular, constantly recurring series; and they will repay the trouble of a minute analysis, and of referring them to the conditions on which they depend…But you may fairly ask—is it then at all possible to explain the whole of the events described on the assumption of…a molecular change as we formerly called it?—Julius F. Cohnheim (1).

Since Cohnheim's meticulous *in situ* observations of the microvascular events that occur in inflamed tissues (1), generations of researchers have been directing their efforts at understanding the molecular changes he had postulated. Activation cascades are thought of as a key principle here; many of Cohnheim's "most heterogeneous agencies" can activate cells within tissues that activate cells of the vascular wall that activate blood-borne leukocytes, and inflammation commences. But what, exactly, does "activation" mean in this context? What, for instance, defines the activation state of a neutrophil? A widely held assumption is that a neutrophil's life is determined by a one-dimensional program. Circulating cells are quiescent *a priori* and do not require constant reminders that this is how they are supposed to be. Any signal, physical or chemical, that triggers a change in behavior may be seen as a wake-up call capable of instigating inflammation.

Findings by Moazzam *et al.* (2) reported in this issue of the *Proceedings* suggest that the simple dichotomy of resting versus activated may be insufficient to describe a neutrophil's condition. The authors found that neutrophils became more deformable and retracted pseudopodia when exposed to a surface shear stress between 0.06 and 1 dyne/cm², which is in the low range of shear that is physiologically encountered in the blood stream. Because inflammatory stimuli typically induce stiffening and spreading in neutrophils (3, 4), traditionally we would interpret the opposite reactions exhibited by neutrophils upon exposure to fluid shear as a remission to a less activated state. However, Moazzam *et al.* (2) also observed that the neutrophil response to shear depended on the opening of calcium and other ion channels, and longer exposure to shear led to cell swelling. Calcium flux is a hallmark response elicited by a plethora of mediators that are considered neutrophil activators (5). Similarly, chemoattractants induce rapid neutrophil swelling, and this increase in volume is necessary before cells can migrate toward a chemotactic stimulus (6). Thus, the mechanisms and consequences of neutrophil exposure to shear bear qualities that have been traditionally associated with both the induction and reversal of activation.

The suggestion that the fluid dynamics of a neutrophil's intravascular environment may provide a constant modulating signal that activates some subroutines of the behavioral program while inhibiting others opens a number of possibilities. Because neutrophils must rapidly adhere to and migrate across the endothelial lining of physiologically perfused venules in inflamed tissues, it seems unlikely that the signals associated with shear have a mere paralyzing effect. In fact, a minimum shear is required for neutrophil rolling mediated by selectin adhesion molecules (7). However, it appears conceivable that some qualities of neutrophil behavior may be down-regulated in response to the hemodynamic forces. Moazzam *et al.* (2) show that two of these qualities are the cell's polarization and stiffness.

What might be the physiologic consequences of a shear-induced increase in neutrophil deformability? As long as a cell moves freely in the laminar blood stream, the average shear stress that it encounters on its plasma membrane depends on the difference in the velocity of its fastest moving part closest to the vessel axis and the slowest part facing the vessel wall (8). For a buoyant cell this shear stress is very small in larger vessels, but increases incrementally during its voyage through successively narrower branches of the arterial tree. As the cell enters the microcirculation, a shear stress may be encountered that is even higher than that employed in the study by Moazzam *et al.* (2). In nonpulmonary tissues, where leukocyte diameters are larger than those of capillaries, leukocytes are frequently observed to hesitate briefly at capillary ostia before they continue their journey (Fig. 1). This delay reflects the time required for the cells to deform. Although the necessary deformation can be considerable, leukocytes that become permanently stuck within capillaries were shown to be very rare, even when the capillary lumen was further reduced because of postischemic swelling of endothelial cells (9). A leukocyte that is stuck in a capillary ostium while its trailing end points into the lumen of a terminal arteriole may be exposed to a shear stress of up to about 100 dyne/cm² (10). Could this sudden exposure to high shear hasten and/or enhance the deformation process via the processes described by Moazzam *et al.* (2)? Such a mechanism may help explain why leukostasis in postischemic capillaries was found to be a rare event unless the systemic perfusion pressure and, consequently, the shear rate was lowered to levels that usually are only encountered in the low pressure circulation of the lung (10). Accordingly, mechanical sequestration of leukocytes has been observed in the lung (3), but appears to be rare in other organs. The continuous kneading of circulating neutrophils in nonpulmonary organs may thus provide the means to facilitate their unimpeded passage through capillaries and improve microvascular perfusion and gas exchange.

Shear-induced modulation of other leukocyte functions also seems plausible, although speculative at present. For instance, neutrophil-derived histotoxic agents such as oxygen radicals and proteases are necessary to defend an organism against invaders. However, this armamentarium is a two-edged sword, because uncontrolled release of these and other mediators may damage tissues (11). One of the tissues that is most exposed and, thus, vulnerable to a misguided attack is the vasculature itself. It seems reasonable, therefore, that some sort of safety net may have evolved to keep leukocytes from secreting mediators at the wrong place or an inappropriate time. A number of protective factors, for example, intra- and extracellular antioxidants (12, 13), or endogenous protease inhibitors (14, 15) come to mind. Their job is containment and
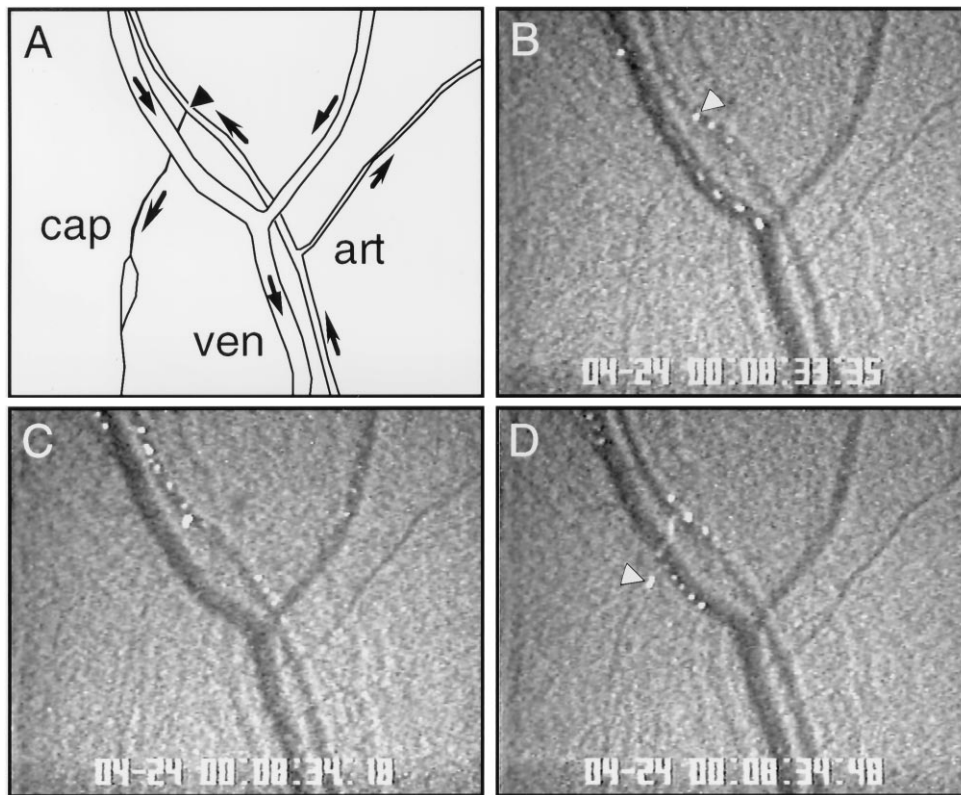

FIG. 1.   Delayed entry of neutrophils into a rabbit mesentery capillary. (*A*) A schematic drawing depicts the microvascular anatomy; an arteriole (art) and a branched venule (ven) can be seen. The ostium of a capillary (cap) branching off the arteriole is indicated by an arrowhead. The direction of blood flow is indicated by arrows. (*B*) Intravital micrograph of fluorescently labeled neutrophils. Cells were visualized by stroboscopic epi-illumination, which causes the appearance of fast-moving fluorescent cells as bright spheres with several fainter after-images. One neutrophil (white arrowhead) has just become stuck in the capillary's ostium. (*C*) The cell is visibly deformed 0.73 s after it has become stuck. Blood flow in the downstream capillary has come to a complete stop, whereas flow in the arteriole remains unobstructed. This is evident because several other neutrophils in the arteriole are passing the stuck cell without apparent delay. The neutrophil's trailing end, which remains in the arteriolar lumen, is exposed to an estimated shear stress of 40 dynes/cm^2. This fluid shear may confer a signal to the cell that may enhance its deformability so that it can eventually pass the capillary entrance. (*D*) After a period of 1.13 s since the neutrophil had initially stopped, it has deformed sufficiently to enter the capillary and continue its passage without further delay.

neutralization of neutrophil-derived mediators, whereas other pathways are known to prevent neutrophil activation. These include signals that raise intracellular levels of cAMP in neutrophils such as prostaglandin E-1 and -2 (16). Adenosine (17), antioxidant vitamins (18, 19), and constitutively generated nitric oxide (20) are similarly protective. These moderators of acute inflammation are more or less ubiquitous, whereas fluid shear stress is a unique feature of the intravascular compartment. What better way for a neutrophil, then, than to use this circumstance to determine whether its current whereabouts are intra- or extravascular, and to postpone an all-out assault on its surroundings as long as shear is present? Although Moazzam *et al.* (2) did not test this concept, they have established that one of its prerequisite requirements has been met: neutrophils can rapidly detect and react to fluid shear.

Although the idea that neutrophil responsiveness to shear may have evolved as a protective mechanism seems appealing, many challenges to this concept remain to be answered and the influence of shear on other qualities of neutrophil function must be examined. Do different shear levels promote qualitative or quantitative differences in the neutrophil response to proinflammatory mediators? What are the effects of shear on oxidative burst and degranulation? How does a neutrophil sense shear, and what are the signaling pathways that are involved? Is there a shear-response element that regulates transcriptional activity in neutrophils as has been shown for endothelial cells (21, 22)? Can we demonstrate a change in perfusion and/or damage to microvessels by manipulating the

neutrophils' ability to detect fluid shear? Are there clinical scenarios in which these events might play a role? If so, there may be opportunities for new therapeutic approaches that may prevent uncontrolled neutrophil activation in conditions such as septic or hypovolemic shock, where high levels of proinflammatory mediators and low flow are seen in the peripheral circulation.

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