How monocytes guard the glomerulus

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In most tissues the leukocyte response to inflammatory stimuli takes place in postcapillary venules (1). Leukocytes and endothelial cells undergo a coordinated and stereotyped series of increasingly tight adhesive interactions designed to capture leukocytes from the bloodstream (2–5). This process generally culminates in migration of leukocytes across the endothelial cells lining the postcapillary venules en route to the site of inflammation. Some notable exceptions occur in specialized capillary beds, such as those that supply the pulmonary alveoli (6, 7) and those that comprise the renal glomerulus (8, 9). Hickey and colleagues recently used intravital microscopy to study the behavior of neutrophils and monocytes in murine glomeruli in the presence and absence of inflammation (9). The authors reported that neutrophils and monocytes normally spend extended periods of time (several minutes) stationary or crawling slowly within the renal capillary lumen. In the presence of local inflammatory stimuli, the duration of these interactions (dwell time) increased and the leukocytes showed signs of intravascular activation (9). In PNAS the group takes these observations to the next level, providing some mechanistic insight into these phenomena (10). The Finsterbusch et al. study sheds some light on how the inflammatory response is regulated in the glomerulus, and also raises some interesting questions for future research.

The Role of “Nonclassical” Monocytes

First, the monocytes that spend time interacting with the glomerular capillaries are overwhelmingly the “nonclassical” subset (10). These cells, sometimes called “patrolling monocytes,” are best referred to by the surface markers that they display when isolated. In the mouse, these cells express high levels of the fractalkine receptor CX₃CR1 and low or undetectable levels of the chemokine receptor CCR2 and the myeloid marker Ly6C, and are referred to as CX₃CR1^{hi} CCR2[−] Ly6C[−]. In contrast, “classical” monocytes, sometimes called “inflammatory monocytes,” express nearly the opposite surface phenotype. These monocytes are CCR2^{hi} CX₃CR1^{lo} Ly6C⁺ (11–14). (The human counterparts of

nonclassical monocytes are CD14^{lo} CD16^{hi}; of classical monocytes CD14^{hi} CD16[−].) These two monocyte subsets have different migratory phenotypes, different roles in the inflammatory response, and tend to mature into different types of macrophages.

The nonclassical subset gained notoriety for their “patrolling” behavior in postcapillary venules of the dermis and mesentery (12). There, rather than flow past the vessel wall or roll on it transiently like neutrophils or classical monocytes, these cells maintained prolonged contact with the vessel wall migrating back and forth along it, but rarely trans-migrating. In the glomerular capillaries, CX₃CR1^{hi} monocytes display similar prolonged contact (9, 10), and similarly, this behavior is partially dependent on CX₃CR1 and integrin CD11a/CD18 (10, 12), although the combination of CD11a/CD18, CD11b/CD18, and CD49d/CD29 are required for maximally prolonged contact in the glomerulus. These similarities may be surprising considering the anatomic and physiologic differences between the vessel types. Postcapillary venules at sites of leukocyte extravasation are 40 to >60 μm in diameter, so leukocytes have to actively adhere to the endothelial lining to avoid being swept away in the bloodstream (15, 16). Capillaries are about 8 μm in diameter, considerably smaller than the diameters of monocytes (~20 μm) or neutrophils (~13 μm), which have to deform as they squeeze tightly through the capillary beds. This raises the question of whether the nonclassical monocytes in the glomerulus are actively patrolling or being passively retained. Obviously, it is not merely a question of size, because: (i) classical monocytes are essentially the same size and show much less of this behavior, (ii) the number of monocytes displaying this behavior is markedly reduced when leukocyte integrins are blocked, and (iii) the number of neutrophils displaying this behavior is markedly reduced in the absence of monocytes (10). The question then becomes, why do classical monocytes not display this behavior, because they have to squeeze through the same capillary beds? The answer may lie partially in the presence or absence of CX₃CR1

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on the leukocytes. Endothelial cells express fractalkine (CX₃CL1), a transmembrane chemokine (17), on their surfaces. When the ligand engages its receptor, CX₃CR1 on leukocytes, it triggers activation and adhesion of the leukocytes (18). Indeed, ablation of the fractalkine gene, *Cx3cr1*, significantly reduces monocyte patrolling in glomeruli (10). However, the effect is not complete, indicating that there are other factors that promote this behavior in CX₃CR1^{hi} monocytes.

Monocyte–Neutrophil Interactions

Second, neutrophils also show this static and migratory behavior within glomerular capillaries and it is increased and prolonged in the presence of inflammation induced in the glomerulus by immune complexes in the glomerular basement membrane (GBM) (9). The new data show that monocytes are required for efficient “patrolling” behavior of neutrophils. Ablation of monocytes using clodronate liposomes reduced neutrophil numbers in the glomerulus by two-thirds and their dwell time by about 50% (10). This degree of depletion had physiologic consequences, because monocyte-depleted mice were protected from anti-GBM glomerulonephritis, a neutrophil-dependent inflammatory disease (10).

However, the manner in which monocytes were eliminated produced different results. Clodronate liposomes deplete monocytes and macrophages systemically. This treatment resulted in a considerable reduction in patrolling neutrophils in the glomerulus and a similar reduction in their dwell time. However, the number of activated neutrophils was reduced proportionally. That is, the proportion of activated neutrophils in the inflamed glomerulus was not altered when all susceptible monocyte and macrophage populations were reduced. In contrast, when CX₃CR1^{hi} (nonclassical) monocytes were selectively eliminated, even though the numbers of monocytes were reduced, the numbers of neutrophils in the inflamed glomerulus and their dwell times were not decreased, yet proportion of activated neutrophils was decreased by 50%. The authors hypothesize that this is a result of the ability of CX₃CR1 in monocytes signaling to promote neutrophil activation independent of its ability to recruit them (10). This explanation is quite plausible, but there are at least two other nonmutually exclusive possibilities: CX₃CR1 is also expressed, albeit at much lower levels, on neutrophils. It is possible that this receptor plays a role in directly activating neutrophils in the inflamed glomerulus in which CX₃CL1 expression on the glomerular endothelial cells is elevated. Alternatively or additionally, there may be effects on these inflammatory events carried out at a distance by classical monocytes and macrophages that are important, even if these cells are not in the glomerulus.

Third, monocytes and neutrophils make physical contact in the glomeruli. These interactions are prolonged during anti-GBM nephritis and associated with increased neutrophil activation (10). Activation was assessed by fluorescence of dihydroethidium in the neutrophils, a marker of oxidation produced by reactive oxygen species. However, the contact itself was not what activated the neutrophils. Careful examination showed that one-sixth of the neutrophils that became activated never contacted a monocyte, and half of the activated neutrophils that did contact monocytes became activated before contacting the monocyte. Furthermore, depletion of monocytes with clodronate liposomes only reduced the number of activated neutrophils (as measured by dihydroethidium fluorescence) by half. What then is the physiologic significance of monocyte–neutrophil

contact? Is it just increasing the likelihood of neutrophil activation? Is it just an epiphenomenon? Or is it performing some other yet unknown function?

The Role of Soluble Mediators

Fourth, TNF- α produced by the CX₃CR1^{hi} monocytes in response to inflammation was primarily responsible for the activation of neutrophils in the anti-GBM glomerulonephritis model (10). Anti-TNF antibody reduced proteinuria by about 50%. This treatment had no effect on monocyte or neutrophil stasis in the glomerular capillaries nor on monocyte dwell time. It did, however, reduce neutrophil dwell time by about half and reduced the number of activated (dihydroethidium-positive) neutrophils

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by >80% (10). Thus, TNF- α produced by nonclassical monocytes in the glomerulus in response to immune complexes in the GBM activates neutrophils in their vicinity, which are responsible for most of the damage to the glomerulus in anti-GBM nephritis. The activation may be augmented by direct contact, but does not need to be.

Specialized Inflammation in the Glomerular Capillary Bed

These experiments made use of sophisticated intravital imaging using both multiphoton and spinning-disk confocal microscopy. The former can penetrate deep into tissues, allowing observation of events in solid organs like the kidney in real time with minimal disturbance of the native tissue. The latter imaging method is generally much faster, allowing multiple rapid measurements that accurately track the positions of rapidly moving cells, such as leukocytes in three dimensions. However, to image glomeruli by spinning-disk confocal microscopy requires special preparation of the kidney (9). The authors show that they obtain similar results with either system (9, 10), which increases confidence in the validity of the results.

In most inflammatory settings the stimulus is outside the blood vessel. In postcapillary venules, leukocytes—including neutrophils—become activated enough to adhere to and transmigrate the endothelial cells and subendothelial basement membrane, but not enough to activate their NADPH oxidase. Full activation is kept in check until neutrophils reach their target (5). In contrast, in glomerulonephritis, the inflammatory stimuli are immune complexes and complement in and on the GBM. Monocytes and neutrophils do not have to transmigrate to reach the inflammatory stimulus. They can contact it by inserting pseudopods between glomerular endothelial cells. Therefore, just as the molecular interactions to recruit myeloid cells to sites of inflammation in specialized capillary beds may differ from those in postcapillary venules, so may the activation and effector steps, especially when the inflammatory stimulus is within the capillary vascular bed. Future experiments using direct observation of the capillary bed will undoubtedly increase our knowledge of the regulation of inflammation in the glomerulus, as well as shed light on the interactions of the various leukocyte subsets that interact in both the innate and adaptive immune response.

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