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Angiophagy: Clearing or Clogging Microvessels?

Costantino Iadecola

Feil Family Brain and Mind Research Institute, Weill Cornell Medical College, New York, NY 10021, USA

Abstract

The body clears small clots from blood vessels through a process called angiophagy, opening up new approaches to combat cerebral and cardiac microvascular occlusive diseases (Grutzendler *et al.*, this issue).

Microemboli (usually small blood clots, but also cholesterol crystals and fragments of atherosclerotic plaques) can be generated during long plane trips, cardiac arrhythmias, or medical procedures (1–3) and must be cleared from the circulation to avoid microvascular occlusions and tissue damage. Hemodynamic forces, which push the emboli downstream, and fibrinolysis, which dissolves clots, are critical mechanisms of disposal of these emboli. In a recent study, it was shown that endothelial cells also play a role in clearance by engulfing microemboli and removing them from brain microvessels, in a process termed "angiophagy" (4). In this issue of *Science Translational Medicine*, Grutzendler *et al.* (5) report that angiophagy reopens occluded microvessels in humans as well as in mice and in organs other than the brain. However, such endothelial engulfment of clots can also prevent their clearance by hemodynamic forces and fibrinolysis. Thus, angiophagy can promote retention or removal of emboli, depending on whether the process is in the early (immediately after embolization) or later stages.

WHAT HAPPENS TO MICROEMBOLI?

Previously, Lam and colleagues used in vivo two-photon microscopy to investigate the fate of microemboli (8 to 20 μm) that had been introduced into the cerebral circulation of mice (4). A large number of emboli were cleared from the vessels; however, unexpectedly, the removal was attributable not only to washout by hemodynamic forces or fibrinolysis but also to the ability of endothelial cells to "grab" and translocate the emboli from the vessel lumen to the perivascular space (Fig. 1). The translocation process took several days to complete and was less efficient in aged mice. The authors' initial appraisal of the biological importance of this phenomenon was that it was beneficial, leading to recanalization of occluded microvessels and preventing ischemic brain injury. In addition to its homeostatic function in the healthy mouse brain, failure of recanalization in aged animals suggested new mechanisms for microvascular occlusive diseases in the elderly (4).

Corresponding author. coi2001@med.cornell.edu.

Key questions remained unanswered in that study (4). It was unclear whether angiophagy was operative also in other organs, particularly the heart and lungs, which are frequently threatened by emboli. The early temporal dynamics of the process were also unclear because imaging studies were conducted 24 hours after the embolization. Last, and importantly for translation, it was not known whether this mechanism of embolic clearance was also present in humans.

In the present study (5), Grutzendler *et al.* set out to address these questions using mice expressing green fluorescent protein (GFP) in endothelial cells (*Tie2-GFP*). The cerebral microcirculation was examined in vivo in these mice by using two-photon microscopy, whereas the cardiac, pulmonary, and renal microcirculations were examined in fixed tissue. In the brain and heart, only ≈50% of the emboli were cleared 1 day after embolization, presumably through hemodynamic washout and/or fibrinolysis. By examining the relationship between emboli and the vessel wall, the authors found that endothelial cells extended processes (filopodia, lamellipodia) that enveloped the embolus, keeping it “stuck” to the vessel wall and preventing its clearance by hemodynamic forces (Fig. 1).

The clot-busting drug tissue plasminogen activator (tPA) improved the clearance of the clots, but the effect became modest if the administration was delayed by 6 hours after embolization—a finding that is consistent with clinical observations that tPA is most efficacious within 3 to 4 hours after thromboembolic events (6). Immunocytochemical studies suggested that the endothelial processes enveloping the embolus also restricted the access of circulating tPA to the clot, reducing its thrombolytic efficacy (Fig. 1). Because endothelial cells produce their own tPA, however, it remains unclear why locally produced tPA would not be aiding in clot lysis. One possibility is that angiophagy up-regulates the endogenous tPA antagonist plasminogen activator inhibitor 1 (PAI1), suppressing local fibrinolytic activity. Irrespective of the role of tPA, these observations by Grutzendler and colleagues indicate that in the first few hours after embolization, in the brain and in other organs, angiophagy promotes emboli retention and may account for the partial efficacy of washout and fibrinolysis in clearing the microvascular bed of emboli (5).

The benefits of angiophagy became evident by imaging 24 hours after embolization in mice. Previously occluded vessels in the brain, kidneys, and heart opened up, and flow was fully reestablished, without evidence of vascular or tissue damage (4). The recanalization of vessels that were occluded with fibrin clots (≤50 μm) occurred over the course of 1 to 6 days, whereas cholesterol microemboli were slower to clear, vascular patency being reestablished in 3 to 8 days. Studies with mice expressing GFP in pericytes or macrophage/microglia revealed that the microemboli, after going through endothelial cells, were engulfed by pericytes, intramural vascular cells enriched in capillaries, and then translocated into the perivascular space, in which they were phagocytized and degraded by microglia/macrophages (5).

In contrast to fibrin clots, cholesterol emboli were not degraded. Cholesterol emboli are a frequent cause of retinal ischemia in humans, and a retrospective analysis of retinal fluorescein angiograms in patients provided evidence that extravasation of cholesterol emboli may also occur in humans (5). Remarkably, in one patient the extravasation was

associated with resolution of retinal ischemia, attesting to the overall beneficial effects of angiophagy. The discovery that angiophagy may also occur in humans attests to its translational possibility as a target for human thromboembolic diseases.

UNIVERSAL CLEARANCE

Angiophagy occurs not only in brain and retina but also in the heart, lung, and kidneys, although the temporal characteristics of the extravasation and the cells involved in the perivascular disposal are different. Whereas in the heart and kidney, the time course of the extravasation was similar to that of the brain, in the lung, the process was slower (6 to 11 days)—which Grutzendler *et al.* attributed to the fact that alveolar macrophages engulfed the emboli while still in the blood vessel lumen, seemingly slowing down the speed of extravasation. However, macrophages were also involved in angiophagy in the kidney, yet the temporal characteristics of the phenomenon were similar to the brain, where macrophages did not play a prominent role. The bases for these temporal discrepancies and their pathobiological implications remain unclear; their elucidation would require organ-specific studies.

Angiophagy is not the only process for extravasation and perivascular translocation of biological material; hematogenous tissue invasion by leukocytes, tumor cells, and parasites are other examples. Leukocyte extravasation has been extensively investigated in brain as in other organs, and its cellular and molecular features are relatively well understood (7). The process begins with selectin-mediated rolling of the cells along the endothelial surface, followed by more secure attachment at particular sites of the endothelial membrane enriched with projections expressing specific adhesion receptors (7). The cell then exits the vessel lumen either through the endothelial cell body (transcellular route) or between adjacent endothelial cells (paracellular route). The vascular basement membrane is then locally disrupted at specific sites to allow the exit of the cell from the blood vessel wall (7). This highly orchestrated sequence of events is implemented by the spatially and temporally coordinated expression of a complex array of adhesion receptors, ligands, cytokines, chemokines, and proteases (7). Similar—but often molecularly distinct—mechanisms are involved in the extravasation of circulating tumor cells and parasites (8, 9).

Unlike the processes of extravasation described above, the molecular effectors underlying angiophagy remain to be established, although metalloproteases (MMPs) have been implicated (4). Rearrangement of the endothelial cytoskeleton and microtubules leading to filopodia and lamellipodia extensions, coupled to expression of adhesion receptors and interactions of the emboli with the extracellular matrix, are likely to be involved. Furthermore, platelets, which are critical in other extravasation modalities, may also play a role in facilitating the attachment of the emboli on endothelial cells. These possibilities need to be tested experimentally.

BRIGHT AND DARK SIDES OF ANGIOPHAGY

Could angiophagy be a therapeutic target for diseases associated with embolic microvascular occlusions? There is a great clinical need to develop strategies to counteract the devastating

impact of microembolic cardiac and cerebral diseases, which are major causes of morbidity and mortality worldwide. Particularly, age-related brain diseases in which microvascular occlusions play a role, such as stroke and dementia (10), could benefit immeasurably from approaches to prevent or reduce the impact of microvascular occlusions through enhanced angiophagy. This indication is of particular interest, considering that angiophagy is attenuated by aging (4). Furthermore, the lack of tissue reperfusion after endovascular procedures for large artery recanalization—the so-called “no reflow” phenomenon attributed to microvascular occlusions (2)—would benefit from enhanced angiophagy.

However, the dichotomous nature of angiophagy unveiled by Grutzendler *et al.* (5) suggests caution. On the one hand, inhibition of angiophagy in the first few hours after embolism could be of clinical value because it would facilitate the vascular clearance by hemodynamic forces and fibrinolysis. On the other hand, promoting angiophagy in the late phase after microembolic events may be beneficial because it would enhance the removal of microvascular occlusion and reestablish tissue perfusion. Our understanding of the molecular bases of angiophagy is still rudimentary, and a more complete picture of the cellular and molecular bases of the phenomenon is needed, especially in humans, in order to develop targeted approaches that selectively increase the transmigration of emboli without damaging vascular function or affecting blood-brain barrier permeability. Targeting the different phases of angiophagy would require reliable biomarkers. This would be challenging if the embolic source is intermittent, such as in atrial fibrillation, because the process would be in different stages of development in different vessels at any given time.

Irrespective of these challenges, the findings of Grutzendler *et al.* (5) shed further light on an intriguing phenomenon in both mice and humans that is likely to be of vital import for maintaining the integrity of the vasculature and organ homeostasis. In an era in which microvascular embolic diseases are increasingly common owing to lifestyle, aging, and medical procedures, the data provide insights into endogenous homeostatic mechanisms that could be instructive in developing new treatments for these crippling conditions.

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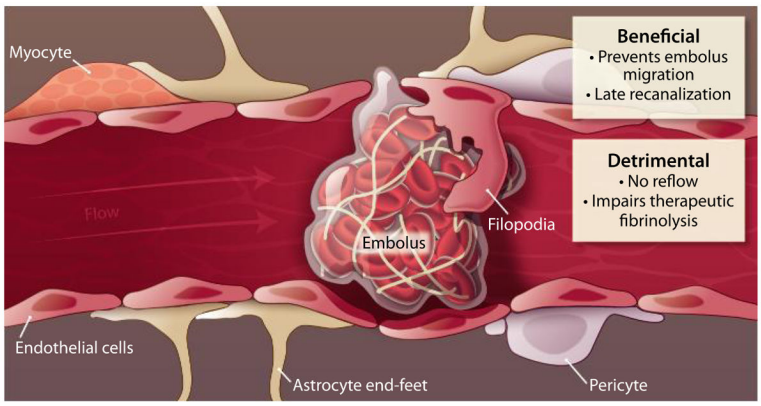

Fig. 1. Clearing versus clogging

Angiophagy is an endogenous mechanism for the clearance of microemboli, such as clots and cholesterol crystals, from arterioles. A cerebral arteriole is depicted here, but angiophagy occurs also in other organs beside the brain, according to image-based findings from Grutzendler *et al.* (5). In angiophagy, emboli are first engulfed by filopodia protruding from endothelial cells. Eventually, the embolus is transported through the vessel wall into the perivascular space and degraded by phagocytic cells (not shown). Angiophagy could be beneficial immediately after embolization by preventing downstream migration of emboli that may completely occlude smaller vessels and, in the chronic setting, by leading to recanalization of occluded microvessels. Conversely, angiophagy could be detrimental by preventing the washout of emboli by hemodynamic forces (no-reflow phenomenon) and by making the engulfed portion of the embolus less accessible to the clot-busting action of tPA.