Role of caveolin-1 in regulation of inflammation: different strokes for different folks

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The article by Hu et al. (10) describes a novel role for caveolin-1 in regulating antimicrobial responses of neutrophils as well as inflammatory lung injury. Previous reports had documented expression of caveolin-1 in immune cells (9), including professional phagocytes such as neutrophils (38), but its functional role was not well understood. Hu et al. provide compelling evidence for the pivotal role of caveolin-1 in regulating neutrophil functional responses that underpin innate immunity. How then does caveolin-1, a ubiquitously expressed protein with well-recognized roles in protein and cholesterol trafficking in endothelial cells and in tumor suppression, modulate the activation of these itinerant leukocytes? To understand this, it is important to review our current understanding of the functions of caveolae and caveolins.

Caveolae ("little caves"), so named because of their appearance by transmission electron microscopy, are flask-shaped invaginations in the plasma membrane 50–100 nm in diameter that lack a membrane-dense coat (21, 37). They are present in most cells but are particularly plentiful in cells of the cardiovascular system such as endothelial cells and vascular smooth muscle cells as well as in adipocytes and fibroblasts. Caveolae have been implicated in a wide variety of cellular events including transcytosis of proteins and cholesterol trafficking and more recently as signaling platforms that regulate diverse cellular processes (1).

The best-known function of caveolae is in endocytosis, a process by which extracellular substances such as albumin are engulfed into free membrane vesicles that bud from the plasma membrane and are then internalized and targeted to specific intracellular compartments. Endocytosis involves complex mechanisms including membrane fusion events that are integral to intracellular trafficking of vesicles. Notably, the classical endocytotic pathway involves clathrin-coated pits, whereas caveolae are thought to participate in a parallel endocytic pathway. However, recent evidence indicates that these pathways intersect and that caveolin-1 confers long-lasting stability to caveolar vesicles that then interact with endosomes and selectively release cargo into these structures (23).

Interestingly, certain microbial pathogens such as simian virus 40 and certain strains of Escherichia coli have evolved mechanisms to exploit these processes to gain entry into eukaryotic cells through caveolae (18, 28).

As implied by their name, caveolins are proteins intimately associated with caveoleae (26). Three caveolin genes are expressed in mammals (CAV-1, CAV-2, and CAV-3), which encode four protein isoforms (caveolin-1α and -β, caveolin-2, and caveolin-3) (31). Caveolin-1, also known as caveolin, Cav-1, or VIP21, was the first member of the caveolin family to be identified as a 22-kDa tyrosine phosphorylated protein in cells transformed by the Rous sarcoma virus and was established to be a structural component of caveolae and of transport vesicles derived from the trans-Golgi network (7, 26). Caveolin-1 is ubiquitously expressed, albeit at different levels in specific tissues. The highest levels of expression of caveolin-1 are in adipocytes, endothelial cells, fibroblasts, smooth muscle cells, and type I alveolar epithelial cells. Caveolin-2 is tightly coexpressed with caveolin-1, and the latter is required for the proper membrane localization and stability of caveolin-2. By contrast, caveolin-3 is expressed predominantly in striated muscle cells such as in the heart and skeletal muscle (32).

All three caveolins tend to form oligomeric complexes of between 14 and 16 monomers that are dependent on a conserved structural motif known as the caveolin domain (27).

Much has been learned about the functions of caveolins by the generation and study of mice deficient in each of the caveolin genes. In general, these mice are viable, but each knockout has characteristic phenotypes attributable to the mutant gene. For example, caveolin-1-deficient mice display elevated endothelial nitric oxide synthase (eNOS) activity in vascular endothelial cells and enhanced susceptibility to carcinogen-induced tumor formation (2, 4, 24). Mice deficient in caveolin-1 or caveolin-2 demonstrate severe pulmonary abnormalities including disorganization and thickening of the alveolar walls with hypercellularity attributable to endothelial proliferation and pulmonary fibrosis (4, 25). Mice deficient in either caveolin-1 or caveolin-3 develop severe cardiac hypertrophy that is much more severe in mice deficient in both caveolin-1 and -3. Mutations in caveolins have also been implicated in human diseases. For example, mutations in CAV-1 are associated with breast cancer (30), and mutations in CAV-3 result in rippling muscle disease and a type of limb-girdle muscular dystrophy (15, 34).

The functional importance of caveolins in immune and inflammatory responses is just beginning to be elucidated. For example, small interfering RNA knockdown of endothelial caveolin-1 impairs the transcellular route of transendothelial migration of leukocytes (14), and mice genetically deficient in caveolin-1 display attenuated microvascular sequestration of neutrophils and lung injury in response to challenge with intraperitoneal lipopolysaccharide (LPS) (6). In this model, caveolin-1, via regulation of eNOS-derived nitric oxide production, appears to be a crucial determinant of NF-κB activation and consequent pulmonary inflammation in response to LPS. There is also evidence that caveolin-1 plays a key role in antigen-presenting cells, leading to activation of T lymphocytes (19), and in modulating macrophage activation (35).
The study of Hu et al. (10) contributes to this emerging literature and addresses the functional importance of caveolin-1 in neutrophils, professional phagocytic cells that are pivotal in host defense but under pathological circumstances may contribute to inflammatory tissue injury (16, 17). The authors studied functional responses of bone marrow neutrophils from caveolin-1-null mice. These studies revealed that caveolin-1-null neutrophils are defective in agonist-induced oxidant production, adhesion, and transendothelial migration compared with wild-type neutrophils. Furthermore, using an isolated, perfused murine lung model in which caveolin-1 null or wild-type neutrophils are perfused through wild-type (caveolin-1 sufficient) lungs, the authors demonstrate that caveolin-1-null neutrophils, activated with the combination of formyl-Met-Leu-Phe (fMLP) and platelet-activating factor (PAF), induce less microvascular injury than wild-type neutrophils. How can we relate these functional anomalies to the known roles of caveolin-1?

Some clues to these mechanisms can be found in the literature describing the role of caveolae as lipid-based signaling platforms that both compartmentalize and concentrate signaling molecules (the “caveolae signaling hypothesis”) (5). Indeed, caveolae can be viewed as a type of lipid raft, which are highly ordered microdomains located within the plasma membrane enriched in certain lipids (29). Recent studies have underscored the importance of caveolins including caveolin-1 as negative regulators of diverse cellular signaling pathways. It is important to recall that while caveolins are located primarily at the plasma membrane, they are also present in the Golgi, in the endoplasmic reticulum, in vesicles, and within the cytosol, thus extending the potential reach of their signal-regulating influence (36). On the basis of its predicted amino acid sequence and mutational analysis, caveolin-1 is envisaged to have a membrane-spanning hairpinlike structure with both the amino and carboxy termini directed toward the cytoplasm (22). Specific motifs within the caveolin proteins serve to recruit lipids and proteins to caveolae, thus facilitating intracellular trafficking of cellular machinery and regulation of signaling pathways. A wide assortment of signaling molecules including heterotrimeric GTP-binding protein subunits, small GTPases, receptor and nonreceptor tyrosine kinases, and eNOS bind caveolin-1 through its “caveolin scaffolding domain” (CSD) (3, 11, 12). In many of these interactions, caveolin-1 appears to dampen signaling pathways by inhibition of many proteins including c-Src, H-Ras, mitogen-activated protein (MAP) kinases, and eNOS (12, 20).

The study by Hu et al. (10) provides additional mechanistic data linking caveolin-1 to activation of Rac1 and Rac2, small GTPases known to act as molecular switches in agonist-induced pathways leading to antimicrobial responses of neutrophils such as activation of the NADPH oxidase, chemoattractant-induced actin reorganization, and migration (8). Furthermore, overexpression of recombinant caveolin-1 in “engineered phagocytes” (Cos-phox cells) resulted in enhanced chemoattractant-induced superoxide production, providing strong evidence for a signal-enhancing role of caveolin-1 in this response.

As for any novel study, more questions are raised than are answered. For example, how does caveolin-1 facilitate Rac1/2 activation involved in triggering antimicrobial responses? In this regard, it is known that both Rac1 and components of the NADPH oxidase colocalize in caveolin-1-containing caveolae (33). It can be envisioned that in these signaling platforms caveolin-1 maintains Rac in an active conformation (40), thus promoting and enhancing oxidant production. Whether this involves modulation of the activities of guanine nucleotide exchange factors (GEFs) or guanine nucleotide dissociation inhibitors (GDI s) is unknown and an obvious direction for future studies. Another issue that should be addressed is whether part of the function of caveolin-1 in neutrophil activation is related to vesicle fusion events analogous to those involved in caveolae-dependent endocytosis. Many antimicrobial responses in neutrophils including phagocytosis, exocytosis of granule contents, assembly and activation of the NADPH oxidase, adhesion, and motility involve vesicle fusion events between the plasma membrane, nascent phagosomes, endosomes, granules, and secretory vesicles (39).

In summary, the study by Hu et al. provides important insight into a novel signal-enhancing role for caveolin-1 in regulation of effector responses of neutrophils that are central in combating invading microbial pathogens as well as in mediating inflammatory tissue injury. From these and other recent observations, it is apparent that caveolae should join the ranks of other multimolecular signaling platforms like lipid rafts and focal adhesions (13, 29). These signaling platforms represent a potential target for novel therapeutics designed to prevent or ameliorate inflammatory tissue damage in conditions such as acute lung injury, sepsis, ischemia-reperfusion injury, and arthritis.

REFERENCES


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