The expanding roles of caveolin proteins in microbial pathogenesis

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Caveolin proteins have been implicated in a wide range of cellular functions including lipid raft-mediated endocytosis and regulation of cell signaling cascades. Recent discoveries have shown that these proteins are involved not only in regulating these homeostatic cellular functions, but also in the host response to a wide range of different infections. Both caveolin-1 and 2 have been shown to play important roles in pathogen uptake. While caveolin-1 is the most well-studied member of this family, a growing body of evidence has now recognized the role of caveolin-2 in these host-pathogen interactions and novel host-defense mechanisms.

Introduction

Caveolae are specialized lipid raft domains which are composed of cholesterol, glycosphingolipids, GPI-anchored proteins and caveolin proteins. The role of caveolae is increasingly being recognized to play an important role in the pathogenesis of a wide range of infectious diseases. A variety of pathogens including parasites, viruses and bacteria have evolved mechanisms of invading host cells in order to survive and replicate. Caveolin proteins have been implicated in not only pathogen uptake by host cells but also in coordination of the inflammatory response to these different microbes. Some of the different pathogens that have been reported to require caveolae or caveolin expression are shown in Table 1. While most of the literature on the role of caveolin proteins in microbial pathogenesis has focused on caveolin-1, we have discovered a previously unrecognized role of caveolin-2 in these host-pathogen interactions. This review will focus specifically on the role of caveolin proteins in microbial pathogenesis and the expanding roles of caveolin-2 as a key determinant of the host-pathogen interactions.

Members of the Caveolin Family

The caveolin family consists of three different proteins: caveolin-1, caveolin-2 and caveolin-3. In addition to serving as key structural proteins that organize caveolae platforms, caveolin proteins are important in regulating endocytosis and cell signaling. Caveolin-1 and -2 are co-expressed on most cell types including high levels of expression on the endothelium as well as type I pneumocytes within the alveolar epithelium. Caveolin-2 is the most divergent of the caveolin gene family with only approximately 50% similarity to caveolin-1. Caveolin-2 interacts with caveolin-1 to form a hetero-oligomeric complex within lipid rafts. Unlike caveolin-1, caveolin-2 is not required for the formation of caveolae and cannot form caveolae by itself. In order for caveolin-2 to be expressed, caveolin-1 is required to transport caveolin-2 to the plasma membrane. In the absence of caveolin-1, caveolin-2 is not expressed since a majority remains in the cytoplasm and is degraded in the Golgi. This dependence of caveolin-2 on caveolin-1 expression has confounded the studies aimed at distinguishing the function of these two proteins.

Role of Caveolae and Caveolin-1

Caveolae were recognized to constitute an alternative pathway of endocytosis and one of the first descriptions of caveolin-1 in bacterial infections was its ability to influence the endocytic uptake of pathogens. Caveolin-1 was recognized to play a key role as a structural protein in the formation of the flask-shaped caveolae that line the plasma membrane of numerous cell types. Functional studies of caveolae have used agents such as nystatin, filipin or methyl-β-cyclodextrin to disrupt cholesterol enriched lipid rafts as well as microscopy to show co-localization with caveolae-associated proteins such as caveolin. A wide range of pathogens have been shown to require lipid rafts and co-localize with markers of caveolae in order to invade host cells. On the other hand, only a small number of bacterial pathogens have been shown to specifically require caveolin expression for entry into host cells. Caveolin-1 was shown to be required for the uptake of not only viral pathogens such as Simian Virus-40, and BK virus, but also the physically larger bacterial pathogens. Initial studies of Escherichia coli entry into both mast cells and bladder epithelial cells showed caveolae dependent endocytosis was a mechanism for bacteria to invade both phagocytic and non-phagocytic cells. Intracellular bacteria were co-localized with caveolin-1 and invasion was inhibited by agents that disrupted the integrity of caveolae by removing membrane cholesterol. E. coli uptake not only required the organization of lipid rafts, but siRNA experiments demonstrated that bacterial invasion was specifically dependent on caveolin-1 expression. Campylobacter jejuni is a leading cause of invasive diarrheal illnesses whose...
invasion of intestinal epithelial cells is dependent on caveolin-1 expression.\textsuperscript{16} Despite the numerous differences in the pathogenesis of these highly diverse infections, caveolin-1 is required for pathogen invasion of host cells.

Caveolin-1 has also been shown to be an important cell signaling protein that acts as an inhibitory regulator of endothelial nitric oxide synthase and its role in modulating the inflammatory response. Through its scaffolding domain, caveolin-1 has been implicated as an inhibitor of these inflammatory signaling cascades. More recently, studies of the caveolin-1 knockout (cav1\textsuperscript{-/-}) mouse have confirmed the importance of caveolin proteins using in vivo models of infection. Using an intraperitoneal model of sepsis, it has been shown that cav1\textsuperscript{-/-} mice are resistant to lung injury and have decreased mortality due to the lower levels of inflammation mediated by the interactions with nitric oxide.\textsuperscript{17} On the other hand, murine models of salmonella infection have shown that cav1\textsuperscript{-/-} mice have higher levels of inflammatory cytokines and increased mortality after challenge with \textit{Salmonella typhimurium}.\textsuperscript{18} Since the cav1\textsuperscript{-/-} mice lack expression of both caveolin-1 and 2,\textsuperscript{7,19} the distinct roles of the caveolin proteins in these in vivo models are not yet known. Both of these studies highlight the importance of caveolin proteins as not only a regulator of pathogen invasion, but also the host inflammatory response to different pathogens.

### Recent Discoveries of the Role of Caveolin-2

While the numerous functions of caveolin-1 have been studied extensively, the role of caveolin-2 is less well understood. Caveolin-2 has not previously been recognized as a regulator of lipid raft mediated endocytosis or cell signaling pathways.\textsuperscript{10} The dependence of caveolin-2 on caveolin-1 has confounded the studies aimed at distinguishing the function of these proteins. Our recent study of pseudomonas pneumonia has helped distinguish the specific role of the caveolin proteins by selectively overexpressing caveolin-1 and 2. \textit{Pseudomonas aeruginosa} is a major cause of hospital acquired pneumonia and a significant cause of hospital mortality.\textsuperscript{20,21} Although previously considered an extracellular pathogen, it has been shown that Pseudomonas is capable of invading both airway as well as alveolar epithelial cells during the pathogenesis of pneumonia.\textsuperscript{5,22,23} Using a murine pneumonia model, we have shown that cav1\textsuperscript{-/-} mice are highly resistant to Pseudomonas.\textsuperscript{6} Similar to many of the pathogens described above, Pseudomonas invasion is significantly decreased by disruption of lipid rafts.\textsuperscript{5,22} RNA interference to inhibit caveolin-1 expression was effective at decreasing Pseudomonas invasion but reduces expression of both caveolin-1 and 2.\textsuperscript{5} Using overexpression vectors, we have subsequently shown that caveolin-2 and not caveolin-1 is responsible for the regulation of Pseudomonas invasion of lung epithelial cells.\textsuperscript{6} The mechanism by which caveolin-2 regulates Pseudomonas uptake involves a complex interaction with members of the src family of tyrosine kinases including C-terminal src kinase (Csk) and c-src.\textsuperscript{6} We have described a novel host defense mechanism whereby in response to infection host cells inhibit the phosphorylation of caveolin-2 thereby inhibiting further entry of the bacterial pathogen. Our studies have revealed that epithelial cells in the lung inhibit the lipid raft mediated entry of Pseudomonas via the interactions of src family kinases with caveolin-2. Csk counteracts the phosphorylation of caveolin-2 triggered after infection by inactivating c-Src. In the absence of Csk, the unregulated phosphorylation of caveolin-2 increases, and lipid raft-dependent internalization of Pseudomonas progresses undeterred, resulting in significant increases in the number of intracellular bacteria. Previously, since caveolin-2 lacks a scaffolding domain, it had not been recognized as a key signaling molecule.\textsuperscript{2,24} We have now shown that caveolin-2 can regulate host endocytosis of bacterial pathogens via its interactions with src family of tyrosine kinases.\textsuperscript{8} The importance of caveolin-2 is not limited to Pseudomonas pathogenesis. \textit{Chlyamidia trachomatis} is believed to invade host cells via a lipid raft dependent mechanism and associates with caveolin-2, but not caveolin-1.\textsuperscript{25} \textit{Rickettsia conorii} is a tick-borne pathogen and the causative agent of Mediterranean spotted fever whose invasion of epithelial cells has been shown to be dependent on caveolin-2 expression.\textsuperscript{26} Although the role of lipid rafts is recognized in a wide range of different infections, the role of caveolin-2 has not yet been investigated in many of these diseases.

### Conclusions

The plasma membranes of mammalian cells are not a simple fluid bilayer, but rather a highly organized series of distinct domains including caveolin enriched lipid rafts. A wide range of pathogens have evolved mechanisms to invade host cells by co-opting these endocytic pathways. Caveolin proteins play an important role by regulating bacterial uptake as well as the host inflammatory response in this ongoing struggle between the host and a range of bacterial, viral and parasitic pathogens. Whereas caveolin-1 has been implicated in bacterial endocytosis and in modulation of the inflammatory response, the role of caveolin-2 has

| Bacteria                     | Caveolin proteins implicated | Role of caveolin |
|------------------------------|------------------------------|------------------|
| \textit{Pseudomonas aeruginosa}| Cav-1 & Cav-2               | Invasion         |
| \textit{Chlamydia trachomatis}| Cav-1 & Cav-2               | Invasion         |
| \textit{Salmonella typhimurium}| Cav-1                       | Inflammation     |
| \textit{Escherichia coli}    | Cav-1                       | Invasion         |
| \textit{Listeria monocytogenes}| Cav-1                       | Invasion         |
| \textit{Campylobacter Jejuni}| Cav-1                       | Invasion         |
| \textit{Mycobacteria species}| Cav-1                       | Invasion         |
| \textit{Group A Streptococci}| Cav-1                       | Invasion         |
| \textit{Anaplasmaphagocytophilum}| Cav-1                     | Invasion         |
| \textit{Ehrlichia chaffeensis}| Cav-1                       | Invasion         |
| \textit{Shigella flexneri}   | Cav-1                       | Invasion         |
| \textit{Francisella tularense}| Cav-1                       | Invasion         |
| \textit{Brucella species}    | Cav-2                       | Invasion         |
| \textit{Rickettsia}          | Cav-2                       | Invasion         |
remained obscure. One problem in identifying the unique role of caveolin-2 has been the dependence of caveolin-2 on caveolin-1 expression. Recently, we showed we could overcome this impediment and revealed an important role for caveolin-2 in the pathogenesis of Pseudomonas pneumonia. Interestingly, our studies also revealed a counteractive novel host cell mediated mechanism that actively blocked bacterial entry. Thus successful invasion of Pseudomonas depends on which of these forces prevails. A better understanding of these early events in the pathogenesis of infection will hopefully allow for the development of new treatments targeting these host cell signaling pathways to treat and prevent these deadly infections.

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