Microbial pathogenesis: **Lipid rafts as pathogen portals**

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The route of initial entry influences how host cells respond to intracellular pathogens. Recent studies have demonstrated that a wide variety of pathogens target lipid microdomains in host cell membranes, known as lipid rafts, to enter host cells as an infectious strategy.

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Current Biology 2000, 10:R823–R825

0960-9822/00/$ – see front matter

Pathogenic microbes have evolved myriad strategies to evade immunological defenses and secure a protected niche within their host. Some avoid ingestion by the phagocytic cells designed to degrade them, while others promote their uptake and reside within 'safe' compartments inside host cells, protected from antimicrobial agents elsewhere in the cell. It is becoming increasingly apparent that the mechanism by which microbes enter cells can impact their intracellular survival. In a recent paper, Shin *et al.* [1] report that *Escherichia coli* bacteria expressing the FimH adhesin enter mast cells through specialized regions of the host plasma membrane called 'lipid rafts' [1]. Recent studies have demonstrated that these lipid microdomains are also the target for other pathogens, including viruses, parasites and prions (Figure 1).

Lipid rafts

The lipid microdomains that are known as lipid rafts can be found in both the plasma and endosomal membranes of eukaryotic cells. These dynamic regions are characterized by detergent insolubility, light density and enrichment for cholesterol, glycosphingolipids and GPI-linked proteins that are anchored in the membrane by their attached glycosyl-phosphatidylinositol lipids (reviewed in [2,3]). Caveolae are a subset of lipid rafts at the plasma membrane that are characterized by their 'flask-like' morphology. In addition to the above biochemical criteria, caveolae are also defined by the presence of the cholesterol-binding protein caveolin, which forms a coat around the plasma membrane invagination [4].

Lipid rafts appear to have many functions, although their complete roles are not well understood. These functions include such diverse processes as polarized secretion, membrane transport, transcytosis across epithelial monolayers and the generation of cell polarity [3,5,6]. The importance of lipid rafts in signal transduction is highlighted by their enrichment for many signalling molecules such as receptor tyrosine kinases, mitogen-activated protein (MAP) kinases, adenylyl cyclase and lipid signalling intermediates [2]. Although lipid rafts comprise only a small percentage of the cell surface area, their high concentration of signalling molecules makes them a natural target for microbes to communicate with the host cell. Lipid rafts are also known to undergo fission from the plasma membrane, mediating a form of endocytosis that is different from clathrin-coated pit internalisation [7]. Hence, microbial agents might also favor interaction with lipid rafts as a potential way to enter host cells.

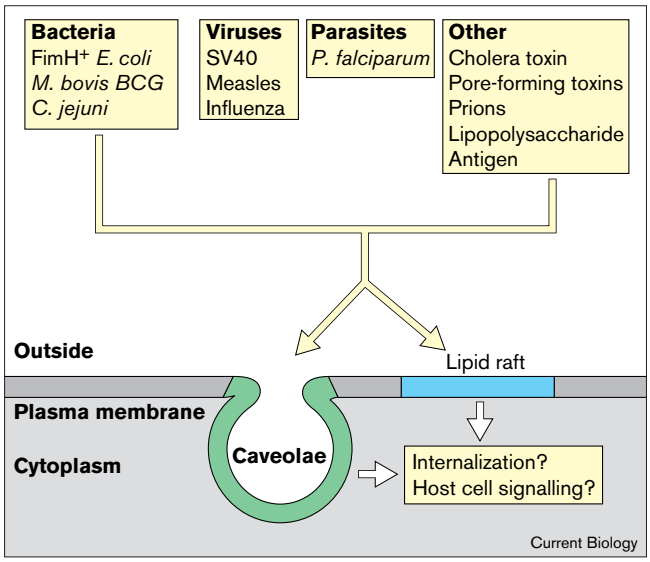
Bacterial entry via lipid rafts

Until recently, expression of the bacterial FimH adhesin presented a paradox. This *E. coli* surface molecule binds sugars on host cells to permit adherence to and colonisation of mucosal surfaces, a key step in establishing infection. On the other hand, FimH also mediates adhesion to phagocytes, such as macrophages, that normally ingest and kill bacteria. Perhaps adding meaning to this confusion, Baorto *et al.* [8] have demonstrated that FimH mediates bacterial uptake into macrophages in a manner distinct from antibody-mediated phagocytosis, and that this allows for bacterial survival within these cells.

In their recent study, Shin *et al.* [1] have demonstrated that CD48, a GPI-linked receptor for FimH on the host cell surface [9], is present in lipid rafts of mast cells. These raft domains contain the caveolae marker caveolin, though it should be noted that flask-shaped caveolae have not been detected in these cells. Using a combination of colocalisation and inhibitor studies, Shin *et al.* [1] obtained evidence that lipid rafts are essential docking points for bacterial entry following FimH interaction with its receptor. Importantly, FimH-expressing bacteria were co-purified with these caveolae-like lipid rafts from infected cells. These findings suggest that FimH mediates entry into phagocytes by a novel mechanism that allows their survival in a lipid-raft-containing vacuole, and provides a mechanism by which *E. coli* can become an intracellular pathogen.

Recent studies have also implicated lipid rafts in the uptake by mouse macrophages of the intracellular pathogen *Mycobacterium bovis BCG* [10]. The mycobacteria bind cholesterol with high affinity and cholesterol-disrupting agents blocked uptake of the bacteria, suggesting that the binding is relevant to internalisation of the pathogen. Association of the macrophage's tryptophane-aspartate-containing coat protein (TACO) with the cytoplasmic face of mycobacterial phagosomes is thought to block interaction

Figure 1



Pathogens and their components target lipid rafts. Lipid rafts and caveolae are microdomains within host cell membranes that are targeted by various pathogens, including bacteria, parasites, and viruses. Pathogens and their components may interact with lipid rafts to promote internalization or communication with the host cell through the many signalling molecules enriched at these sites.

of this compartment with lysosomes [11]. TACO binding to phagosomes was found to be cholesterol dependent, perhaps providing a general mechanism by which lipid-raft-internalized pathogens can avoid normal endocytic processing [10]. Another bacterial pathogen which may enter host cells by lipid rafts is *Campylobacter jejuni*, as cholesterol chelators that disrupt caveolae were found to inhibit their entry into an epithelial cell line [12].

Binding and/or uptake of some soluble bacterial products also occurs at lipid rafts. For example, the cholera toxin B subunit binds to its receptor GM_1 in these microdomains, possibly allowing for the concentration of individual toxin subunits within the plane of the membrane [13]. The pore-forming toxin Aerolysin from *Aeromonas hydrophila* is also targeted to lipid raft domains [14]. CD14, a co-receptor for the bacterial cell-wall component lipopolysaccharide, has been localized to low density regions of the plasma membrane, a characteristic of lipid rafts [15]. It would be interesting if the Toll-like family of 'pattern recognition' receptors, which are known to bind various microbial products, are also localized to lipid rafts.

Other pathogens

Viruses target lipid raft microdomains during viral entry into cells, as well as during viral assembly before budding out of cells. SV40 is the best-characterized virus that targets lipid rafts during infection. The SV40 virus co-opts these lipid microdomains to enter host cells and reach its niche in the endoplasmic reticulum. The non-enveloped virus SV40 binds a receptor on host cells, the GPI-linked class I major histocompatability complex (MHC) molecule, which is enriched in caveolae. Virus particles that enter the host cell are associated with multiple markers of cellular caveolae, and caveolae-disrupting drugs inhibit viral entry [16,17]. The measles and influenza viruses appear to assemble at these domains, and rafts purified from infected cells are infectious as they contain all of the components of a mature virion [18,19].

There is also growing evidence to support the hypothesis that the pathogenesis of prion disease is localized to caveolae-like domains. A region of the GPI-linked cellular prion protein targets it specifically to caveolae, and this is an essential step in the conversion of the prion protein into the pathogenic isoform. The restriction of pathogenic prion formation to this subcellular compartment indicates that other caveolae-enriched molecules are likely to be involved in this pathogenic process [20]. Caveolae-like structures have also been reported in the membranes surrounding the parasite *Plasmodium falciparum*, the causative agent of malaria [21].

Biological significance

While caveolae and other lipid microdomains have been identified as sites of microbial action, the biological consequence of these interactions requires further investigation. Caveolae may be targeted by pathogens if they offer access to a protected intracellular niche. Previous studies have demonstrated that *E. coli* entering macrophages via the FimH adhesin reside within a compartment that permits bacterial survival by resisting both acidification and the release of toxic reactive oxygen species [8]. Furthermore, *Mycobacterium bovis BCG* can avoid fusion of its vacuole with lysosomes in macrophages [11]. The interactions between lipid-raft-derived vacuoles and the endosomal system are poorly understood, so it remains to be determined how pathogens residing within these compartments manage to avoid the host's antimicrobial arsenal.

While some pathogens exploit lipid rafts for entry, these domains may also serve as 'trip wires' that can be triggered by a pathogen to initiate an inflammatory response by the host. For example, recognition of bacterial FimH by its receptor CD48 triggers secretion of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) by mast cells [9]. Mast cells are strategically located at sites of pathogen entry into the host, and their secretion of TNF-α is necessary for recruitment of other immune cells and clearance of bacterial infection [22]. Caveolae are involved in the uptake of respiratory syncytial virus antigen by dendritic cells in a mechanism distinct from other antigen uptake pathways [23]. While caveolae-mediated uptake of this soluble antigen leads to activation of an immune response, it is not known if lipid-raft-derived vacuoles containing pathogens interact with the antigen-processing pathway.

It is likely that lipid-raft-mediated signalling can be either beneficial or detrimental to the host cell, depending on the pathogen that is encountered. Such a scenario is reminiscent of the phagocytic M cells from Peyer's patches in the intestine: while these cells are essential for sampling of lumenal antigens by underlying lymphoid tissues, the trade-off is that Peyer's patches are a target for many invasive pathogens. Further study of how microbes interact with lipid rafts on the host cell surface are likely to teach us a great deal about how microbial pathogens set up their intracellular niche and how the host cell utilizes these multipurpose signalling centers to respond quickly to infection.

Acknowledgements

We thank members of the Finlay lab for critical review of this manuscript. Work in B.B.F.'s laboratory is supported by operating grants from the Canadian Institute for Health Research and the Canadian Bacterial Diseases Network. C.M.R. is supported by a studentship from the Natural Sciences and Engineering Research Council of Canada. J.H.B. is supported by a postdoctoral fellowship from CIHR and is an honorary fellow of the Killam Memorial Foundation. B.B.F. is a Howard Hughes International Scholar and a CIHR scientist.

References

1. Shin JS, Gao Z, Abraham SN: **Involvement of cellular caveolae in bacterial entry into mast cells.** *Science* 2000, **289:**785-788.
2. Anderson RG: **The caveolae membrane system.** *Annu Rev Biochem* 1998, **67:**199-225.
3. Simons K, Ikonen E: **Functional rafts in cell membranes.** *Nature* 1997, **387:**569-572.
4. Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG: **Caveolin, a protein component of caveolae membrane coats.** *Cell* 1992, **68:**673-682.
5. Anderson RG: **Caveolae: where incoming and outgoing messengers meet.** *Proc Natl Acad Sci USA* 1993, **90:**10909-10913.
6. Manes S, Mira E, Gomez-Mouton C, Lacalle RA, Keller P, Labrador JP, Martinez AC: **Membrane raft microdomains mediate front-rear polarity in migrating cells.** *EMBO J* 1999, **18:**6211-6220.
7. Parton RG, Joggerst B, Simons K: **Regulated internalization of caveolae.** *J Cell Biol* 1994, **127:**1199-1215.
8. Baorto DM, Gao Z, Malaviya R, Dustin ML, van der Merwe A, Lublin DM, Abraham SN: **Survival of FimH-expressing enterobacteria in macrophages relies on glycolipid traffic.** *Nature* 1997, **389**:636-639.
9. Malaviya R, Gao Z, Thankavel K, van der Merwe PA, Abraham SN: **The mast cell tumor necrosis factor alpha response to FimH-expressing *Escherichia coli* is mediated by the glycosylphosphatidylinositol-anchored molecule CD48.** *Proc Natl Acad Sci USA* 1999, **96:**8110-8115.
10. Gatfield J, Pieters J: **Essential role for cholesterol in entry of mycobacteria into macrophages.** *Science* 2000, **288:**1647-1650.
11. Ferrari G, Langen H, Naito M, Pieters J: **A coat protein on phagosomes involved in the intracellular survival of mycobacteria.** *Cell* 1999, **97:**435-447.
12. Wooldridge KG, Williams PH, Ketley JM: **Host signal transduction and endocytosis of *Campylobacter jejuni*.** *Microb Pathog* 1996, **21:**299-305.
13. Parton RG: **Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae.** *J Histochem Cytochem* 1994, **42:**155-166.
14. Abrami L, Fivaz M, van der Goot, F: **Adventures of a pore-forming toxin at the target cell surface.** *Trends Microbiol* 2000, **8:**168-172
15. Wang PY, Kitchens RL, Munford RS: **Bacterial lipopolysaccharide binds to CD14 in low-density domains of the monocyte-macrophage plasma membrane.** *J Inflamm* 1995, **47:**126-137.
16. Parton RG, Lindsay M: **Exploitation of major histocompatibility complex class I molecules and caveolae by simian virus 40.** *Immunol Rev* 1999, **168:**23-31.
17. Norkin LC: **Simian virus 40 infection via MHC class I molecules and caveolae.** *Immunol Rev* 1999, **168:**13-22.
18. Manie SN, Debreyne S, Vincent S, Gerlier D: **Measles virus structural components are enriched into lipid raft microdomains: a potential cellular location for virus assembly.** *J Virol* 2000, **74:**305-311.
19. Scheiffele P, Rietveld A, Wilk T, Simons K: **Influenza viruses select ordered lipid domains during budding from the plasma membrane**. *J Biol Chem* 1999, **274:**2038-2044.
20. Kaneko K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB: **COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scrapie isoform.** *Proc Natl Acad Sci USA* 1997, **94:**2333-2338.
21. Olliaro P, Castelli F: **Plasmodium falciparum: an electronmicroscopy study of caveolae and trafficking between the parasite and the extracellular medium.** *Int J Parasitol* 1997, **27:**1007-1012.
22. Malaviya R, Ikeda T, Ross E, Abraham SN: **Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α.** *Nature* 1996, **381:**77-80.
23. Werling D, Hope JC, Chaplin P, Collins RA, Taylor G, Howard CJ: **Involvement of caveolae in the uptake of respiratory syncytial virus antigen by dendritic cells.** *J Leukoc Biol* 1999, **66:**50-58.