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Pathogens, toxins, and lipid rafts

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Summary. The plasma membrane is not a uniform two-dimensional space but includes various types of specialized regions containing specific lipids and proteins. These include clathrin-coated pits and caveolae. The existence of other cholesterol- and glycosphingolipid-rich microdomains has also been proposed. The aim of this review is to illustrate that these latter domains, also called lipid rafts, may be the preferential interaction sites between a variety of toxins, bacteria, and viruses and the target cell. These pathogens and toxins have hijacked components that are preferentially found in rafts, such as glycosylphosphatidylinositol-anchored proteins, sphingomyelin, and cholesterol. These molecules not only allow binding of the pathogen or toxin to the proper target cell but also appear to potentiate the toxic action. We briefly review the structure and proposed functions of cholesterol- and glycosphingolipid-rich microdomains and then describe the toxins and pathogens that interact with them. When possible the advantage conferred by the interaction with microdomains will be discussed.

Keywords: Membrane raft; Toxin; Oligomerization; Microdomain; Glycosylphosphatidylinositol; Signaling; Cholesterol.

Abbreviation: GPI glycosylphosphatidylinositol.

Introduction

Pathogens or toxins often exploit components of the target cells, or even hijack a complete existing cellular machinery, to intoxicate or invade their host. The first step in every intoxication or invasion process is recognition of the target cell. This step is generally mediated by specific cell surface molecules that act as receptors. The "choice" of the receptors is the fruit of a long-term coevolution of the bacterium or virus and the host and therefore is believed to favor in one way or another the infection or the intoxication process. A well characterized example is diphtheria toxin which binds to a heparin-binding precursor of an epidermal growth factor(EGF)-like growth factor which addresses the toxin to the endosomal system by internalization via clathrin-coated pits, from where the toxin can enter the cytoplasm and reach its target (Collier 1990). As the number of identified receptors for toxins and pathogens increases, it becomes apparent that each subdomain of the plasma membrane is a preferential site of interaction for a given subset of toxins or pathogens. We will here review the viruses, bacteria, and toxins that have been shown to preferentially interact with cholesterol- and glycosphingolipid-rich microdomains or that were shown to bind to molecules that are enriched in such domains. We will first briefly described the composition and proposed roles of these microdomains and then describe which toxins and pathogens exploit plasma membrane microdomains to potentiate their cytotoxic activities.

Cholesterol-glycosphingolipid-rich microdomains, or rafts

For excellent recent reviews on raft function and structure and on membrane lipid organization see Edidin (1997), K. Simons and Ikonen (1997), Harder and Simons (1997), D. Brown and London (1998), R. Brown (1998), Hooper (1998), Rietveld and Simons (1998), and Jacobson and Dietrich (1999). Briefly, lipid rafts are described as disperse liquid-ordered-phase domains. They appear to be dynamic assemblies to which specific lipids and proteins are selectively sequestered, whereas others are excluded. In particular, they are enriched in glycosphingolipids, choles-

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terol, sphingomyelin, and lipid-anchored membrane proteins, and they are characterized by a light buoyant density and resistance to solubilization by Triton X-100 at 4 °C. This unusual biochemical property has been very useful, although sometimes misleading, to identify proteins that associate with rafts. A subclass of rafts is composed by caveolae. These are flask-shaped invaginations of the plasma membrane containing the transmembrane protein caveolin (for a review, see Parton 1996). Certain cells such as lymphocytes do not express caveolin and therefore also lack caveolae. They still however have plasma membrane rafts (Parton 1996).

Two major functions have been proposed for rafts. They have been proposed to be implicated in the apical sorting of lipids and proteins in polarized epithelial cells (for a review, see Simons and Ikonen 1997), and more importantly in the context of the present review they are thought to compartmentalize, modulate, and integrate signaling events at the cell surface. A variety of signal transduction molecules have been found to be associated with detergent-insoluble membrane fractions (for a review, see Shaul and Anderson 1998). These include G protein-coupled receptors, G proteins, and adenylate cyclase, molecules involved in the regulation of intracellular calcium homeostasis, and their effectors, multiple components of the tyrosine kinase–mitogen-activated protein kinase pathway, and numerous lipid signaling molecules.

Importantly in the present context, raft components appear to have a different surface mobility than other molecules of the plasma membrane (for a review, see Jacobson and Dietrich 1999). This was mainly studied for proteins anchored by glycosylphosphatidylinositol (GPI). Even at steady state in the absence of cross-linking, GPI-anchored proteins were found to exist in clusters at the surface of living cells and this clustering behavior was dependent on cholesterol (Friedrichson and Kurzchalia 1998, Varma and Mayor 1998). A single-particle tracking analysis of the GPI-anchored protein Thy-1, with antibodies conjugated to colloidal gold, showed that more than one third of Thy-1 at the cell surface was temporarily found in so-called confinement zones having a diameter of 260–330 nm (Sheets et al. 1997). This GPI-anchored protein was not found permanently in these zones, illustrating the highly dynamic nature of rafts, but could remain in the zones for up to 9 s. This later property of raft components, i.e., to be at least transiently confined in restricted areas, appears to be crucial for the activity of certain toxins as will be described below.

Bacteria and viruses

The initial interaction of bacterial pathogens with host cells, the first step in many infectious processes, is mediated by a variety of bacterial molecules, called adhesins, which bind specifically to host cell surface components, often called adhesin receptors (for a recent review on host–pathogen interaction, see Finlay and Cossart 1997). A variety of such receptors have been identified and include integral membrane proteins, certain carbohydrate motifs, proteoglycans, but also glycolipids and GPI-anchored proteins. The "choice" of the receptor is however not innocent. In fact, binding to an appropriate receptor not only targets the pathogen to the proper host but often also enables the pathogen to exploit certain useful cellular functions of the host. An example is the recruitment and the rearrangement of the actin cytoskeleton to bacterium–host contact sites either to favor contact or to induce uptake by nonphagocytic cells (Finlay and Cossart 1997). The ability of the pathogen to alter the normal behavior of the host partly relies on its capacity to subvert host signal transduction pathways. Also several signal-transducing molecules have been found to be receptors for bacterial pathogens: integrins were shown to bind enteropathogenic *Escherichia coli*, as well as *Shigella* and *Yersinia* species (for a review, see Cossart 1997), E-cadherin is a receptor for *Listeria monocytogenes* (Mengaud et al. 1996), and the decay-accelerating factor (DAF) binds diffusely adhering *E. coli* (Peiffer et al. 1998). Specific binding of the pathogen to suitable cell surface molecules is critical in determining subsequent pathogen-mediated cellular events. Recent studies have revealed that certain bacteria preferentially associate with rafts or raft components.

Adhering bacteria

Diffusely adhering *E. coli* (DAEC) is a pathogen that adheres to the intestinal epithelium. A DAEC clinical isolate expressing the fimbrial adhesin F1845 was shown to induce microvilli injury and actin disassembly in cultured human intestinal cells (Bernet-Camard et al. 1996). This DEAC clinical isolate was shown to provoke disassembly of F-actin in the apical domain of cells expressing DAF/CD55, a GPI-anchored

protein implicated in the inhibition of complement-mediated lysis (Peiffer et al. 1998). More specifically, binding of DEAC was found to be mediated by the interaction of its fimbrial adhesin F1845 with DAF, and Peiffer et al. (1998) provided some evidence that actin rearrangement is due to signaling through DAF. The precise distribution of DAF on the plasma membrane and the study of its association with signaling pathways were performed in independent studies. Using hematopoietic cells, it was demonstrated that DAF partitions in detergent-resistant membranes, in association with members of the tyrosine kinase Src-like family, such as Lyn and Lck (Parolini et al. 1996). Although direct evidence that DAF signaling occurs via specialized plasma membrane domains is not yet available, there are indications that GPI-anchored proteins are signaling-competent only when they are concentrated in rafts. In an elegant study, Morgan and colleagues indeed showed that CD59 exogenously incorporated into the plasma membrane of CD59-negative promonocyte cells is initially detergent soluble, not associated with kinases, and incompetent for Ca^{2+} signaling upon antibody cross-linking (van den Berg et al. 1995). However, as a function of the time elapsed after incorporation, CD59 acquires the ability to signal, concomitantly to its association with detergent-insoluble complexes and kinases. Importantly the level of Ca^{2+} transients upon cross-linking of exogenously incorporated CD59 after incorporation into microdomains were very similar to those obtained when cross-linking endogenous DAF in control cells. It is tempting to speculate that DAEC has chosen to bind to DAF within membrane rafts because rafts provide an ideal platform through which the bacterium can signal and subsequently cause actin disassembly.

Invading bacteria and viruses

Additional indirect evidence suggesting the implication of lipid rafts in bacterial adherence and invasion comes from the observations that bacterium-induced cytoskeleton rearrangements and bacterial uptake require host tyrosine kinase activity (see below). Non-receptor type tyrosine kinases of the Src-family were found to be associated with detergent-resistant complexes in a variety of different cell types (Arni et al. 1996, Parolini et al. 1996, Harder et al. 1998, Ilangumaran et al. 1998). It has recently been proposed that their activity is in fact optimal when these kinases are juxtaposed to raftlike microdomains (Ilangumaran et al. 1999). Moreover, efficient tyrosine phosphorylation of a number of Src-like tyrosine kinase substrates were found to occur only when these substrates were associated with glycosphingolipid-rich domains (Field et al. 1995, 1997; Arni et al. 1996), suggesting that these microdomains may serve as compartments for Src-mediated signaling. The requirement for tyrosine kinase activity for infection has been shown for a number of bacteria. Tyrosine kinase inhibitors were shown to block invasion of *L. monocytogenes* (Cossart 1997) and several *Yersinia* species (Rosenshine et al. 1992). An Src-dependent signaling cascade was found to be implicated in the *Shigella flexneri*-induced reorganization of the host cell cytoskeleton in epithelial cells (Dehio et al. 1995). Overexpression of *src* was indeed shown to stimulate entry of noninvasive shigella mutants (Dehio et al. 1995), whereas overexpression of a dominant-negative form of *src* led to the inhibition of shigella-induced cytoskeletal rearrangements and thereby of bacterial uptake (Dumenil et al. 1998). Interestingly, ectopically expressed *src* was found to be recruited at sites of bacterial entry and colocalized with phosphotyrosine activity (Dehio et al. 1995). These observations, combined with the plasma membrane distribution of tyrosine kinases, raise the possibility that shigellae trigger activation of an Src-signaling pathway through association with rafts.

Also, the invasive pathogen *Salmonella typhimurium* was shown to induce tyrosine phosphorylation of the EGF receptor upon binding to EGF-receptor-expressing cells and to trigger activation of a mitogen-activated protein (MAP) kinase signaling cascade (Galan et al. 1992). In a separate set of data, activated EGF receptor was recently localized to noncaveolar, low-buoyant-density membrane domains (Waugh et al. 1999), and similarly components of the MAP kinase pathway were found to be enriched in cholesterol-rich microdomains (Liu et al. 1997). It will therefore be important in the future to investigate whether bacteria that stimulate tyrosine kinase signaling pathways preferentially interact with host cells via raftlike microdomains.

Finally, uptake of some pathogenic *E. coli* strains was recently shown to be mediated by cholesterol-rich microdomains. *Escherichia coli* strains expressing the lectin FimH were shown to bind to bone-marrow-derived macrophages exclusively through interaction of FimH with the GPI-linked protein CD48 (Tewari et al. 1993, Baorto et al. 1997). Binding of the bac-

terium to this GPI-anchored protein triggers internalization via a tight fitting phagosome which contains caveolin and CD48 (Baorto et al. 1997). The involvement of raftlike domains was further confirmed by the fact that internalization was inhibited after treatment with cholesterol-affecting drugs, which disrupt these microdomains. The authors propose that the bacterium enters via cholesterol-rich microdomains and thereby bypasses the classical route leading to lysosomes, allowing intracellular survival.

Similarly the nonenveloped DNA simian virus 40 was shown to enter cells via noncoated pits that tightly fit around the virus and contain caveolin (Parton and Lindsay 1999). Entry requires binding to surface major histocompatibility class I molecules and this leads, by an unknown mechanism, to the recruitment of caveolin around the virus and the generation of a caveolae-like pit. The virus finally ends up in the endoplasmic reticulum. Maybe, as proposed for the FimH-expressing *E. coli* strains, entry through rafts and caveolae enables simian virus 40 to escape from the degradative pathway.

Toxins

Gram-negative bacterial endotoxin lipopolysaccharide

One of the most potent bacterial toxins is the lipopolysaccharide (LPS) molecule found on the outer membrane of Gram-negative bacteria. LPS binds specifically to the GPI-anchored surface myeloid glycoprotein CD14 (Ulevitch and Tobias 1994). Binding to this receptor is thought to mediate LPS internalization via a non-clathrin-coated pathway (Kitchens et al. 1998) but also to trigger the LPS-induced MAP kinase activation and cytokine production (Solomon et al. 1998). Interestingly, it has recently been shown that all components required to activate the MAP kinase pathway are enriched in cholesterol-rich microdomains (Liu et al. 1997). The fact that LPS binds to GPI-anchored CD14 within rafts enables it to parasitize a signaling pathway as described above for certain adhering and invading bacteria.

Bacterial A-B_5 toxins

A-B_5 toxins constitute a family of multisubunit toxins, including cholera toxin (CT) and pertussis toxin, that are composed of one A subunit, which carries a toxic enzymatic activity, and a pentameric B subunit, which is implicated in the binding of the toxin to the target cell membrane (for a review, see Montecucco et al. 1994). One of the members of this family is CT, which ADP-ribosylates G proteins involved in the control of adenylate cyclase. CT binds to its target cells via the ganglioside GM1, which is present on the entire surface but is concentrated in raft-caveolae-like domains (Parton 1994). The affinity of binding of CT depends on the number of B subunits that bind a GM1 molecule, the affinity being highest when all five B subunits are involved in binding to the membrane. Clustering of GM1 in rafts is likely to favor this high-affinity binding interaction. In addition to increasing the binding efficiency, rafts and caveolae were shown to provide an entry site for CT into the cell. By comparing the behavior of CT and the related *E. coli* heat-labile type II enterotoxin, LTIIb, which distinguish between gangliosides GM1 and GD1 due to differences in their B subunits, Wolf et al. (1998) have shown that these two toxins only trigger cAMP-dependent Cl^--secretory response when the gangliosides are present in detergent-insoluble domains. In agreement, Orlandi and Fishman (1998) found that the disruption of microdomains by the cholesterol-binding drug filipin inhibited CT internalization and accumulation of cAMP.

Therefore, lipid rafts appear to provide a preferential site for the generation of a high-affinity interaction of CT with the target cell, and most probably for other multivalent toxins that bind to gangliosides such as pertussis toxin, as well as an entry route into the cell.

Pore-forming toxins

Pore-forming toxins are secreted by bacteria either to perforate the plasma membrane of surrounding target cells or the membranes of intracellular organelles during cell invasion. These toxins are generally secreted as soluble proteins which diffuse towards the target membrane to which they bind via specific receptors. Multiple monomers must then come together and polymerize into an amphipathic ring-like structure that finally forms a transmembrane pore. Pore formation in the plasma membrane can have multiple consequences (see below) and may lead to cell death. The number of monomers composing the pore varies from 7 for toxins such as aerolysin from *Aeromonas hydrophila* to about 50 in the case of streptolysin O (SLO). Oligomerization is an absolute prerequisite for channel formation. It is therefore crucial that the prob-

ability of encounter between monomers at the cell surface is as high as possible.

A number of receptors for pore-forming toxins have been identified. SLO and the other members of the thiol-activated toxin family are thought to bind to cholesterol (Bhakdi et al. 1996, Tweten 1995) although it has been recently suggested for listeriolysin O that cholesterol might not itself be the receptor but in fact would trigger a conformational change required for oligomerization and channel formation (Harris et al. 1998, Jacobs et al. 1998). The earthworm *Eidenia foetida* protein lysenin, also called eiseniapore, was found to bind specifically to sphingomyelin, a process which was promoted by cholesterol (Lange et al. 1997, Yamaji et al. 1998). *Vibrio cholerae* cytolysin was shown by a liposome-based assay to require cholesterol and sphingolipids for efficient oligomerization and channel formation (Zitzer et al. 1999). Finally, aerolysin from *Aeromonas hydrophila* (Rossjohn et al. 1998) and the insecticidal *Bacillus thuringiensis* Cry 1A δ-endotoxin (Rajamohan et al. 1998) were found to bind to proteinaceous receptors which are however not transmembrane but anchored to the external leaflet of the plasma membrane via a GPI anchor. More specifically Cry 1A was shown to bind to a 120 kDa aminopeptidase N of the midgut of susceptible insects (Hua et al. 1998, Lorence et al. 1997). Aerolysin was found to bind to a variety of GPI-anchored proteins depending on the cell type, including Thy-1 on T lymphocytes (Abrami et al. 1998, Diep et al. 1998, Nelson et al. 1997).

Interestingly, independent studies have shown that all the above mentioned molecules that act as receptors for pore-forming toxins are components of lipid rafts. In fact there is at present no exception, i.e., a pore-forming toxin that would bind to a nonraft component. That binding on living cells occurs preferentially through cholesterol-glycosphingolipid-rich microdomains on living cells was only shown for aerolysin (Abrami et al. 1998, Abrami and van der Goot 1999). We have shown that aerolysin is located in punctate structures at the surface of the target cell and that it is associated, via its GPI-anchored receptors, to detergent-insoluble domains. Disruption of plasma membrane rafts by cholesterol-sequestering drugs led to the redistribution of the receptor-bound toxin all over the plasma membrane, i.e., to its dilution within the plane of the membrane (Abrami and van der Goot unpubl.). Concomitantly to this dispersion of the toxin, oligomerization was dramatically inhibited. Oligomerization could however be partly restored by increasing the toxin concentration. These studies indicate that binding of the toxin to a component of lipid rafts leads to an increase in toxin concentration within restricted areas and thereby promotes oligomerization. These studies also suggest that clustering of receptors renders cells sensitive to lower concentrations of toxin.

These observations are likely to be relevant to other pore-forming toxins, specially those like SLO for which about 50 monomers must come together to form the pore. Pathogenic agents may have evolved to utilize rafts as concentration devices at the cell surface. A similar concentration mechanism could be implicated in the polymerization of the β-amyloid peptide involved in Alzheimer disease (M. Simons et al. 1998) or the conversion of the prion protein to the scrapie-like form (Taraboulos et al. 1995). We believe that our observations, together with the information available for other toxins, also provide functional evidence for the existence of rafts at the surface of living cells.

Channel formation in the plasma membrane can have a number of consequences such as the triggering of signaling cascades as shown for aerolysin, staphylococcal α-toxin, SLO, and listeriolysin O (Grimminger et al. 1997, Krause et al. 1998, Sibelius et al. 1999), or vacuolation of the endoplasmic reticulum as shown for aerolysin (Abrami et al. 1998) and staphylococcal α-toxin (Abrami and van der Goot unpubl.). However, the possible link between channel formation within rafts and these downstream events remains to be investigated.

Conclusions

The study of raft structure and function is still in its early days. It is however already apparent that an increasing number of pathogens and toxins preferentially interact with such specialized regions of the plasma membrane. The interaction with rafts appears to confer a number of advantages to the pathogen or toxin. Pathogens appear to make use of cell surface components that have the inherent capacity to concentrate locally, i.e., in microdomains, either to create high-affinity interactions, as for multivalent toxins, or to oligomerize, as is the case for pore-forming toxins. It is attractive to speculate that cell surface microdomains may serve as concentration platforms for other molecules, including perhaps physiological ligands. It also appears that by binding to rafts, toxins and specially pathogens can parasite the signaling

cascade pathways to which rafts give access. Finally entry into cells via raft-caveolae-like domains may provide means to avoid the degradative pathway.

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