JCB Comment

Anthrax toxin rafts into cells

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Anthrax toxin binds to a plasma membrane receptor and after endocytosis exerts its deadly effects on the cell. Until now, however, the mechanism of initial toxin uptake was unknown. In this issue, Abrami et al. (2003) demonstrate that toxin oligomerization clusters the anthrax receptor into lipid rafts and this complex is internalized via the clathrin-dependent pathway.

Anthrax caused by *Bacillus anthracis* is one of the oldest diseases of cattle and humans known to mankind. Unfortunately, recent events have revived public interest in this menace. Much effort in the past was devoted to understanding the etiology of the disease and to identifying virulence factors (i.e., toxins) that caused the death of target cells. Surprisingly, until recently, little was known about the entry of anthrax toxin into cells. In this issue, a paper by the group of Gisou van der Goot (Abrami et al., 2003) provides evidence for a direct link between anthrax toxin entry and lipid rafts on the surface of the cell. This work is significant for both its medical importance and as a demonstration of how lipid rafts function in biological processes.

The anthrax toxin is a tripartite virulent factor produced by *Bacillus anthracis*. Two subunits, edema factor (EF;* a calmodulin-dependent cyclase) and lethal factor (LF; a metalloprotease that targets MAPK kinases) are responsible for the toxin's virulence by enzymatically modifying substrates in the cytosol. However, they cannot exert their effects without a third 83-kD protein called protective antigen (PA83) that facilitates their entry into the cell. Recently, an integral membrane protein receptor for PA83, termed anthrax toxin receptor (ATR), was identified (Bradley et al., 2001). PA83 bound to the surface receptor is processed by furin family proteases to a 63-kD protein (PA63) that oligomerizes to form a heptameric ring (Petosa et al., 1997). Only after this step can LF and/or EF be bound (Cunningham et al., 2002). The receptor is then internalized into endosomes where at low pH a channel forms, allowing the entry of LF and EF into the cytoplasm of the cell (Mourez et al., 2002). Abrami et al. (2003) now provide an exciting molecular mechanism for the initial steps of anthrax toxin internalization involving receptor interaction with lipid rafts.

Lipid rafts are postulated to be cholesterol- and glycosphingolipid-enriched microdomains of the cell plasma membrane that differ in their physical properties from the rest of the membrane (Simons and Ikonen, 1997). Some membrane proteins associate specifically with these microdomains, whereas others avoid them. Rafts are envisaged as concentrating devices in many signaling processes and intracellular transport pathways. It must be noted that so far there are no biochemical methods for isolation of pure rafts. However, they are operationally defined as entities resistant to solubilization by cold nonionic detergents and found in light (buoyant) fractions upon centrifugation on an equilibrium sucrose gradient (Simons and Toomre, 2000). Abrami et al. (2003) use this operational definition whenever referring to rafts.

One of the major findings of the paper is that ATR bound to heptameric PA63, but not monomeric PA83, is tightly associated with lipid rafts. This interaction can be abolished by the depletion or reduction of the major raft constituents cholesterol and sphingomyelin. The processing of PA83 to PA63 and subsequent heptamerization appears to cluster the ATR into rafts and have important consequences. Normally, endocytosis of the ATR is slow, allowing time for PA83 to be cleaved by furin proteases, only a small fraction of which are present on the cell surface. PA63, but not PA83, is competent to bind the virulence factors EF and LF, and the complex is now rapidly internalized. As the authors state, this makes ATR an ideal receptor for the toxin.

To enter cells, toxins or viruses usually utilize an existing transport pathway. The anthrax toxin does not seem to be an exception and uses for its "egotistic" purposes the intrinsic properties of rafts and raft-associated proteins. Experiments described by Abrami et al. (2003) reveal two new properties of raft-dependent processes. First, oligomerization of a protein may facilitate its association with rafts, and second, this association may accelerate its endocytosis. It is not clear, though, why ATR bound to PA63 should be tightly associated with rafts. Does the heptamerization of PA63 drive this event? The authors address this question by incubating cells with antibodies against PA83 to cluster the ATR in the absence of PA63. Remarkably, cross-linking with antibodies led to the association of PA83-bound ATR with rafts, indicating that oligomerization itself was sufficient to relocalize ATR. This relocalization was cholesterol dependent. As important as these data are, they raise even more questions. Is the oligo-

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*Abbreviations used in this paper: ATR, anthrax toxin receptor; EF, edema factor; LF, lethal factor.

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merization of a protein a general prerequisite for its localization to rafts, or is it a specific property of ATR? Why do other proteins (e.g., the LDL receptor or the transferrin receptor [Harder et al., 1998]) not enter rafts upon clustering? It will definitely be important to determine if oligomerization can be separated from the raft association. Indeed, the entry of the anthrax toxin into cells provides a way to study this.

The authors also take a first step toward understanding why raft-associated heptameric PA63 is internalized much more efficiently than PA83 by identifying the pathway responsible for endocytosis. One possible mechanism for internalizing the heptameric PA63-bound ATR was via caveolae. These are small invaginations on the surface of many cells and are considered as specific forms of rafts (Kurzchalia and Parton, 1999). Recent studies have demonstrated that simian virus SV40 uses caveolae as an endocytotic vehicle (Pelkmans and Helenius, 2002). Surprisingly, although the regulation of endocytosis of anthrax toxin relies on rafts, internalization of the receptor does not occur by caveolae. In another series of experiments, they demonstrated that heptameric PA63 utilizes the classical clathrin-dependent endocytic pathway. In this respect, the endocytosis of ATR resembles that of the B cell receptor, which undergoes ligand-dependent clustering, raft association, and then is internalized via a clathrin-dependent mechanism (Cheng et al., 1999). A fundamental task for the future will be to clarify how internalization of rafts by the clathrin-dependent pathway is regulated. In the case of anthrax toxin, understanding this process could have important medical implications.

Abrami et al. (2003) clearly demonstrate the physiological relevance of raft integrity to toxin effects. In cells incubated with β-methyl cyclodextrin, a reagent that removes cholesterol from the plasma membrane and thus disaggregates rafts, the PA63/LF complex was less efficiently internalized relative to control cells. As a consequence, LF no longer entered the cytoplasm and cleaved the MAPK kinase, MEK1, thus abolishing its toxic effects. Without being overtly optimistic, one can imagine that in the future rafts may be therapeutic targets for drugs against anthrax or other raft-based diseases. The problem to be solved, however, will be to design drugs that disintegrate only a specific subset of rafts. Randomly extracting cholesterol from the cells of an organism or tissue will not be an acceptable cure for anthrax. In summary, the paper by van der Goot's group (Abrami et al., 2003) exposes a new function for lipid rafts in cells and provides another example of how a toxin can usurp a physiologically relevant process for its own purposes.

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