Lipid rafts clustering and signalling by listeriolysin O

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Abstract

Listeriolysin O, the major virulent determinant of Listeria monocytogenes, is known for forming pores on cholesterol-rich membranes. In the present study, we reveal its other facet, rafts clustering. By immunofluorescence microscopy, we show that the glycosylphosphatidylinositol-anchored proteins CD14 and CD24, which normally exhibit uniform distribution on J774 cells, undergo clustering upon treatment with LLO. The non-raft marker transferrin receptor is unaffected by such treatment. Rafts clustering might explain the induction of tyrosine phosphorylation observed on LLO-treated cells.

Introduction

Fifteen years after the formulation of the rafts hypothesis [1–3], considerable debate still persists and the exact structure of rafts remains unclear. Evidence for the existence of lipid rafts was initially based on resistance of the protein and lipid components to solubilization by non-ionic detergents. However, because of potential artifacts inherent in detergent extraction, the precise size and composition of the native rafts is still unclear.

Recent characterizations of rafts relying on fluorescence resonance energy transfer techniques have also led to conflicting outcomes. For instance, some studies indicated clustering of GPI (glycosylphosphatidylinositol)-anchored proteins in resting cells [4,5], whereas other studies have failed to detect such clustering [6,7]. The consensus view from these investigations is that native rafts are rather small and/or dynamic, thus explaining why they have eluded detection by conventional light microscopy. So far, microscopical visualization of rafts has only been accomplished after cross-linking raft components with lectins and/or antibodies. This allowed raft and non-raft components to segregate into detectable micron-sized patches [8]. Even here there appears to be a remarkable lack of consensus on the composition of such clusters since some studies have indicated that raft subtypes exist and that different GPI-anchored proteins tend to segregate into distinct lipid rafts, thus undercutting co-clustering by antibodies [9]. This obstacle notwithstanding, rafts clustering still stands out as one of the most practical prospects for visualizing rafts in living cells. The development of new tools that allow clustering of rafts irrespective of their subtypes should be an important step towards fully exploiting such prospects. In the present study, we demonstrate that LLO (listeriolysin O) is a potent aggregator of lipid rafts which, in contrast with antibodies, can induce co-clustering of different rafts-associated proteins.

LLO is a pore-forming toxin produced by Listeria monocytogenes that opens up the phagosomal membrane, thus enabling the bacteria to escape into the cytosol where it replicates. LLO accomplishes this by binding to cholesterol, then oligomerizing into large complex pores [10].

LLO is known to trigger several signalling pathways in a variety of host cell types. Although some of these signals are due to the influx of Ca²⁺ through pores [11], other mechanisms of signal induction do exist. If preincubated with cholesterol, LLO loses its cytolytic activity but nonetheless binds to host cells and triggers signalling [10,12]. To understand how LLO interacts with cells to induce signalling, we investigated the potential involvement of lipid rafts. A HA (haemagglutinin)-tagged LLO (HA–LLO) or its cholesterol-inactivated form (CL–HA–LLO) was used to this end.

LLO induces clustering of CD14 and CD24 but not the TFR (transferrin receptor)

To characterize the LLO rafts association, J774 cells treated with HA–LLO or CL–HA–LLO were subjected to detergent extraction. Both forms of the toxin were found to copartition with several rafts-associated proteins into detergent resistant membranes (results not shown). Next, we analysed the membrane distribution of LLO on cells in relation to raft marker proteins CD14 and CD24 as well as TRF as a non-rafts marker. Under basal conditions, both raft markers exhibited even membrane distribution (Figures 1A and 1E) as shown before. The TFR, however, displayed a clustered distribution pattern (Figure 1I) even at basal condition, probably due to its concentration in coated pits.

Subsequently, cells were incubated with either HA–LLO or CL–HA–LLO, fixed, before staining for the bound toxin as well as CD14, CD24 or TFR. Figure 1 displays cells treated with CL–HA–LLO. The toxin was distributed into distinct clusters on cell membranes. Interestingly, CD14 and CD24 exhibited clustering on such cells. Such clusters overlapped

Key words: cholesterol, glycosylphosphatidylinositol anchor, lipid raft, Listeria monocytogenes, listeriolysin O, oligomerization.

Abbreviations used: GPI, glycosylphosphatidylinositol; HA, haemagglutinin; LLO, listeriolysin O; TFR, transferrin receptor.

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Figure 1 | Clustering of GPI-anchored proteins by CL–HA–LLO and induction of tyrosine phosphorylation

(A, E, I) Cells were fixed in 4% (w/v) paraformaldehyde, then stained with rat anti-CD14-FITC (A), rat anti-CD24-FITC (E) or rat anti-TFR-biotin followed by streptavidin-FITC (I). In (B–D, F–H and J–L), cells were first incubated with CL–HA–LLO (1 µg/ml) for 10 min at 22°C, washed then fixed in paraformaldehyde before staining for bound toxin (C, G, K) and the indicated standard proteins. (D, H, L) The merger of the CL–HA–LLO and CD14, CD24 and TFR staining respectively. The CL–HA–LLO was stained using mouse anti-HA–biotin and streptavidin Cy3. (M) Activation of tyrosine phosphorylation by HA–LLO and CL–HA–LLO: J774 cells were treated with HA–LLO or CL–HA–LLO for 5 min and lysed in RIPA buffer [10 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2 mM sodium pervanadate and 1% Nonidet P-40 (v/v)]. Lysates were analysed by immunoblotting using anti-phosphotyrosine. Before immunoblotting, the membranes were stained with Ponceau S to confirm equal protein loading.

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LLO either binds directly to the cholesterol in rafts or is indirectly targeted to rafts by the cholesterol bound in solution. The polymerization of rafts-associated toxin monomers then results in the clustering of rafts leading to signal induction.

In conclusion, LLO is a tool with great potential that not only can be used to visualize rafts but also to identify putative raft components as well as signalling pathways mediated through rafts. The use of CL–LLO allows such studies without damaging cellular membranes.

References

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