Reply

As Insall and Machesky point out, the sequence identity between Wiskott–Aldrich syndrome protein (WASP; and N-WASP) and the consensus pleckstrin-homology (PH) domain sequence is low. Not all PH domains match the consensus well: the PH domain from phospholipase C delta, a well-characterized PH domain, is identical in 21 out of 94 amino acid residues, less than the identity with Sos, although still greater than that with WASP. We first proposed that the N-terminal region of WASP and N-WASP contains a PH domain based on its specific association with phosphatidylinositol (4,5)-bisphosphate but not other phospholipids, a feature characteristic of PH domains, and the observation that conserved acid residues can be aligned with those of typical PH domains[1]. However, it could be premature to call the region a 'PH domain' in the absence of structural data.

The N-terminal region of WASP interacts with WIP[2]. Insall and Machesky emphasize the sequence similarity with Ena–VASP family proteins in the 'WH1 domain', but the deletion of residues 1–46 of WASP, which destroys half of the 'PH domain' but does not destroy the sequence of the 'WH1 domain', also results in strong reduction of the interaction with WIP[2]. In addition, the interaction with WIP requires not only intact 'PH' and 'WH1' domains but also more C-terminal sequences (residues 1–170 in WASP are reported to be sufficient). Thus, it is also questionable whether the 'WH1 domain' is really a functional domain.

In conclusion, we now believe that a much broader region, including both the 'PH domain' and the 'WH1 domain', could form a single functional unit that should be appropriately called a 'domain'. The recent discovery of the Src-homology 2 (SH2) domain structure in the N-terminal region in Cb1, which had not previously been thought to be an SH2 domain because of the lack of enough similarity to the consensus SH2 domain sequence, underlines the importance of the structural analysis[3]. We need to accumulate more functional data and determine the three-dimensional structure to resolve this confusion.

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Landing on lipid rafts

The recent *comment* article by Jacobson and Dietrich[1] discussed the interesting issue of the existence and physiological significance of 'lipid rafts'. As highlighted in their article, despite considerable experimental effort, the evidence about such rafts is still equivocal. We would like to suggest, based on recent observations, that certain toxins, bacteria and viruses that bind to molecules identified as components of the proposed lipid rafts might provide experimental tools for characterization of rafts and examination of their physiological significance. As reviewed by Jacobson and Dietrich[1] and others[2–7], the general view on lipid rafts is that they are enriched in glycosphingolipids, cholesterol, sphingomyelin and GPI-anchored proteins. Recent studies have shown that some of these components act as specific receptors for certain multivalent toxins, pore-forming toxins and even pathogens.

Multivalent toxins

Cholera toxin (CT) is a multisubunit toxin comprising a B pentamer involved in target cell binding and a catalytic A subunit that ADP-ribosylates the G protein involved in the control of adenylate cyclase[8]. The receptor for CT is the ganglioside GM_1, which is present on the entire surface but is concentrated in raft-like domains[9]. Each B subunit binds to a GM_1 molecule with low affinity, but the overall binding affinity is increased when all five B subunits bind to GM_1 and would be favoured in areas where GM_1 is concentrated. In an elegant study, Wolf *et al.*[10] compared the behaviour of CT and the related *Escherichia coli* heat-labile type II enterotoxin, which distinguish between gangliosides GM_1 and GD_1 through differences in their B subunits. They found that these two toxins only trigger a cAMP-dependent Cl^- secretory response when the gangliosides are present in detergent-insoluble domains. Using a different approach, Orlandi *et al.* treated CaCo2 cells with the cholesterol-binding drug filipin, which disrupts lipid rafts. Although filipin did not interfere with CT binding, it inhibited CT internalization and cAMP accumulation[11]. These results suggest that lipid microdomains could provide a preferential site for high-affinity interaction of CT with the target cell and a cell-entry route.

Pore-forming toxins

Many pathogenic bacteria secrete pore-forming toxins that perforate either the plasma membrane or intracellular membranes of mammalian target cells. Although these toxins generally have little if any sequence homology, they share a similar mechanism of action[12]. They are secreted by the bacterium as soluble proteins that bind to their target membrane through specific receptors. The toxins then oligomerize into amphipathic ring-like structures that can form a pore in the target membrane. The probability of encounter between monomers at the cell surface influences the efficiency of the oligomerization process. All the receptors identified so far for pore-forming toxins are components of putative rafts. *Streptococcus pyogenes* SLO, as well as all other members of the thiol-activated toxin family, requires cholesterol for channel formation, either because cholesterol is the receptor for these toxins or because it triggers a conformational change required for oligomerization[13,14]. The earthworm toxin lysenin binds to sphingomyelin, and binding is promoted by cholesterol[15,16]. *Vibrio cholerae* cytolysin requires cholesterol and sphingolipids for efficient oligomerization and channel formation[17]. The receptors for *Aeromonas hydrophila* aerolysin[18,19] and the insecticidal *Bacillus thuringiensis* Cry

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1A δ-endotoxin[20] are GPI-anchored proteins. However, evidence for a role for rafts in the oligomerization process only exists for aerolysin. The receptor-bound toxin distributes in punctate structures on the plasma membrane of living cells and is enriched in detergent-insoluble microdomains[19]. We have found that cholesterol-sequestering drugs, that disrupt the structure of rafts, lead to apparent dilution within the plane of the plasma membrane and inhibition of oligomerization (L. Abrami and F. G. van der Goot, unpublished). One model for pore formation by these toxins is that binding to lipid rafts leads to a local concentration that promotes oligomerization. Thus, pore-forming toxins could provide a powerful tool for studying rafts on the surface of living cells.

Endotoxins

Lipopolysaccharide (LPS) could exploit a different characteristic of lipid rafts – the presence of signalling proteins. LPS binds specifically to the GPI-anchored protein CD14[21]. Binding to this receptor is thought to mediate its internalization but also to trigger LPS-induced activation of mitogen-activated protein kinase (MAPK) and cytokine production[22]. All the components required to activate the MAP kinase pathway are enriched in cholesterol-rich microdomains, which could therefore provide an activation mechanism for LPS[23].

Bacteria and viruses

Some bacteria could also use raft domains to enter cells. *E. coli* strains expressing the bacterial lectin FimH bind to macrophages via a FimH–CD48 interaction[24]. Binding to this GPI-anchored protein triggers internalization of the bacterium in a phagosome that contains caveolin and CD48. The authors proposed that the bacterium enters through cholesterol-rich microdomains and thereby bypasses the classical route leading to lysosomes, facilitating intracellular survival. Similarly, the non-enveloped DNA virus SV40 enters cells via non-coated pits that contain caveolin[25].

Future prospects

Although we are still in the early days of lipid-raft structure-and-function studies, it is apparent that several pathogens and toxins interact preferentially with raft components and thus provide interesting tools for studying the dynamics and function of these microdomains. Pathogens seem to make use of cell-surface components with an inherent capacity to concentrate locally, either to create high-affinity interactions for multivalent toxins or to oligomerize, as is the case for pore-forming toxins. It is attractive to speculate that cell-surface microdomains might serve as concentration platforms for other molecules, including physiological ligands. Moreover, some toxins and pathogens that bind to raft components trigger signalling cascades and/or cell uptake and could therefore provide insights into the role of rafts in both signal transduction and clathrin-independent endocytosis.

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