The role of lipid rafts in signalling and membrane trafficking in T lymphocytes

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Journal of Cell Science 114, 3957-3965 (2001)

Summary

Combinatorial association of different lipid species generates microheterogeneity in biological membranes. The association of glycosphingolipids with cholesterol forms membrane microdomains – lipid rafts – that are involved in specialised pathways of protein/lipid transport and signalling. Lipid rafts are normally dispersed in cellular membranes and appear to require specialised machinery to reorganise them to operate. Caveolin-1 and MAL are members of two different protein families involved in reorganisation of lipid rafts for signalling and/or intracellular transport in epithelial cells. T cell activation induces a rapid compartmentalisation of signalling machinery into reorganised rafts that are used as platforms for the assembly of the signalling complex. Costimulatory molecules participate in this process by providing signals that mobilise raft lipids and proteins, and remodel the cytoskeleton to the contact site. As in epithelial cells, rafts are used also as vesicular carriers for membrane trafficking in T lymphocytes. Furthermore, there are potential similarities between the specialised protein machinery underlying raft-mediated processes in T lymphocytes and polarised epithelial cells.

Key words: Lipid rafts, Signalling, Membrane trafficking, T cells

Introduction

The requirement for an extensive repertoire of proteins is evident, given the number of processes performed by the cell. However, the necessity for the large number of different lipid species (>1000) is less clear. For a long time, lipids were thought to play only a passive role as simple building blocks for membranes that delimit intracellular compartments and separate the internal milieu from the extracellular environment. In the fluid mosaic model, cellular membranes were envisaged as disordered uniform bilayers in which lipids moved freely and randomly by lateral diffusion. During the past decade, a new model that accounts for lipid diversity has emerged. This model proposes the existence in biological membranes of lipid microdomains or rafts that have a high sphingolipid and cholesterol content; unlike the loosely packed, disordered phospholipids present in the bulk of membranes, raft lipids are organised in a tightly packed, liquid-ordered manner (Simons and Ikonen, 1997). Fig. 1A shows a current model of raft structure in which sphingolipids, which contain a sphingosine chain and a long, largely saturated, fatty acyl chain, are packed in small membrane structures. In this model, the voids between the hydrocarbon chains caused by the bulky headgroups are filled with cholesterol. The association of cholesterol with sphingolipids promotes phase separation apparently because of favourable packing interactions between saturated lipids and sterol (Brown, 1998; Brown and London, 2000). Together, sphingolipids and cholesterol form microdomains that float in the shorter, unsaturated phospholipids of the bulk membrane (Harder and Simons, 1997). The peculiar organisation of the rafts restricts the access of proteins in such a way that only proteins attached to the membrane by a lipid anchor, such as glycosylphosphatidylinositol (GPI)-anchored proteins or acylated cytosolic proteins, and certain transmembrane proteins can reside in rafts. The majority of integral membrane proteins are excluded (Fig. 1B).

The tight packing of lipids in rafts confers resistance to solubilisation by non-ionic detergents at low temperatures, which allows their isolation as an insoluble membrane fraction (Brown and Rose, 1992). The use of different detergents or temperatures of solubilisation, or immunoadsorption procedures, results in raft fractions that differ in their lipid and/or protein content. This indicates that the insoluble fractions contain distinct types of raft, and this heterogeneity probably reflects the existence of many distinct rafts within the cells (Cerny et al., 1996; Röper et al., 2000; Millán et al., 1999). Combinatorial association of different sphingolipid species with cholesterol probably accounts for raft diversity. Several lines of evidence support the in vivo existence of rafts and argue against the possibility that they are simply artefacts of the detergent solubilisation procedure (Jacobson and Dietrich, 1999). The size of the rafts in vivo has not yet been established, although different techniques give estimates ranging from 25 nm to 50 nm and predict a composition of probably not more than 10 to 30 proteins (Friedrichson and Kurzchalia, 1998; Varma and Mayor, 1998; Pralle et al., 2000). This size increases by coalescence of the rafts upon extraction with detergents or crosslinking of their components.

Rafts are abundant at the plasma membrane but are also found intracellularly in exocytic and endocytic compartments (Dupree et al., 1993; Gagescu et al., 2000; Puertollano et al., 2001). They are mobile, dynamic entities that move laterally along the plane of the plasma membrane and traffic continuously between the plasma membrane and internal compartments (Nichols et al., 2001).

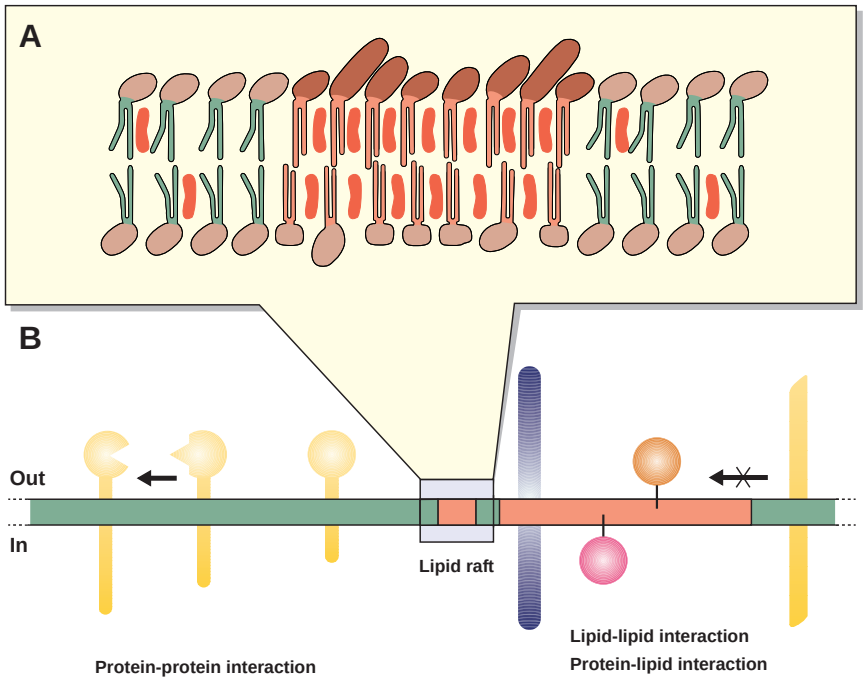


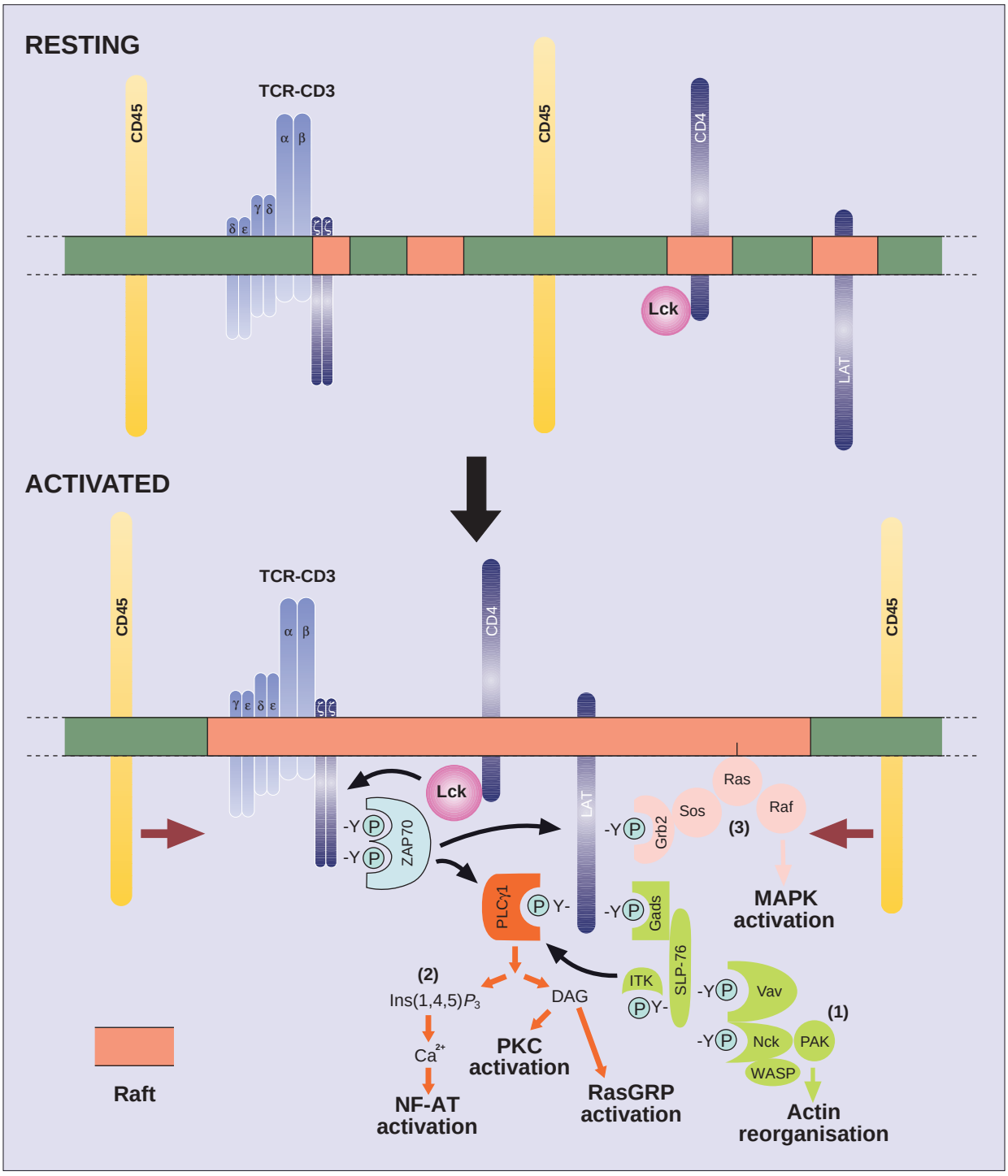
Fig. 1. Model of lipid-raft structure and function in biological membranes. (A) Rafts are membrane microdomains formed by high concentrations of sphingolipids (dark-brown-headed structures) and cholesterol (red bean-shaped structures) immersed in a phospholipid-rich (light-brown-headed structures) environment. Glycolipids and sphingomyelin are restricted to the outer leaflet of the bilayer, whereas cholesterol and phospholipids are in both leaflets. Note that lipids in the rafts usually have long and saturated fatty acyl chains (red two-legged shapes), whereas those in lipids excluded from these microdomains are shorter and unsaturated (green two-legged shapes). (B) Principles of selective recruitment of proteins in rafts. Recruitment of membrane proteins in phospholipid-rich membrane regions takes place through protein-protein interactions. However, in rafts this process takes place through interactions between the lipids within the rafts and the transmembrane domain of integral membrane proteins (lipid-protein interaction) or the lipid moiety of proteins attached to the membrane by a lipid modification (lipid-lipid interaction). The recruitment of cytosolic proteins by protein-protein interactions through modular domains (SH2 domains, SH3 domains, etc.) can take place in both raft and non-raft membranes. Proteins excluded from rafts are in yellow; proteins included in rafts are in blue (integral membrane proteins), light brown (GPI-anchored proteins) or pink (acylated, cytosolically-oriented, proteins such as Src family kinases, Ras and heterotrimeric G proteins).

Rafts as platforms for assembly of the T cell signalling machinery

Pioneering work described the isolation of detergent-resistant membrane complexes enriched in GPI-anchored proteins from T lymphocytes (Hoessli and Rungger-Brandle, 1983). These membranes were then characterised as large non-covalent complexes containing GPI-anchored proteins and Src family tyrosine kinases (Stefanova et al., 1991; Cinek and Horejsi, 1992). The observation that GPI-anchored proteins can transduce activation signals to internal Src family kinases (Bamezai et al., 1989; Gunter et al., 1987; Korty et al., 1991) despite the molecules residing in the opposite leaflets of the lipid bilayer was regarded for a long time as a puzzling and intriguing phenomenon occurring in rafts (Brown, 1993). It is well known that T cell antigen receptor (TCR) engagement triggers the assembly of a large macromolecular complex containing a variety of signalling molecules and adapters, but only recently have investigators postulated that rafts are platforms for this signalling complex (Montixi et al., 1998; Xavier et al., 1998; Zhang et al., 1998). In resting T cells, rafts are highly enriched in the Src kinases Lck and Fyn (Montixi et al., 1998; Xavier et al., 1998) and the linker for activation of T cells (LAT) transmembrane adapter (Zhang et al., 1998). The co-receptor CD4 is detected in raft fractions to a minor extent, and CD3ζ is also partially associated with rafts (Montixi et al., 1998; Xavier et al., 1998) (Fig. 2, top panel). Extensive crosslinking of the TCR with antibodies promotes the rapid activation of Src kinases and subsequent accumulation in rafts of a series of newly tyrosine-phosphorylated substrates (Kane et al., 2000; Langlet et al., 2000; Leo and Schraven, 2001), including virtually all the hyperphosphorylated p23 CD3ζ molecules (Montixi et al., 1998; Xavier et al., 1998; Kosugi et al., 1999), the activated

Fig. 2. Lipid raft reorganisation after TCR engagement. At steady state, CD4, Lck, LAT and CD3ζ are associated with small rafts (red) in T cells. Upon triggering, lipid rafts concentrate in the immunological synapse, gathering together specific membrane proteins. Lck becomes activated and phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) within the CD3 subunits. These phosphorylated motifs become docking sites for the tandem SH2 domains of the tyrosine kinase ZAP70, which is subsequently activated by tyrosine phosphorylation, probably by Lck. Activated ZAP70 phosphorylates the tyrosine residues present in the transmembrane adapter LAT, which recruits Gads, phospholipase Cγ1 (PLCγ1) and Grb2. As a consequence, different processes are triggered: (1) LAT-associated Gads bring the adapter protein SLP-76 to the rafts, and this adapter becomes a substrate for ZAP70. Phosphorylated SLP-76 recruits the Tec family protein tyrosine kinase Itk, the guanine-nucleotide-exchange factor Vav and the adapter molecule Nck. Subsequently, Nck recruits the PAK and WASP proteins through its SH3 domains. PAK and WASP are regulated by Vav and in turn regulate the reorganisation of the cytoskeleton. (2) PLCγ1 recruited to LAT is activated through tyrosine phosphorylation by ZAP70 and Itk. Activated PLCγ1 converts phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P_2) into diacylglycerol (DAG) and inositol (3,4,5)-trisphosphate (Ins(1,4,5)P_3). Subsequently, DAG activates protein kinase C and Ras guanyl-nucleotide-releasing protein (RasGRP), and Ins(1,4,5)P_3 activates the transcription factor NF-AT by promoting Ca^{2+} mobilization and calcineurin activation. (3) Grb2 associated with LAT recruits Sos to the rafts, and this attracts Ras and subsequently other machinery, which results in activation of MAP kinases. Although represented as excluded in resting cells and included in activated cells, the presence in rafts of components of the TCR-CD3 (other than CD3ζ) before and after triggering is controversial (see text). Curved arrows in dark blue indicate relevant tyrosine phosphorylation events occurring upon activation. Horizontal brown arrows indicate tyrosine dephosphorylation events carried out by CD45 molecules present close to the raft edge.

forms of the ZAP70 tyrosine kinase and phospholipase C γ1 (PLCγ1), phosphoinositide 3-kinase (PI3-K), the Vav Rac/CDC42 exchange factor (Montixi et al., 1998; Xavier et al., 1998) and LAT (Brdicka et al., 1998; Zhang et al., 1998) (Fig. 2, bottom panel). The presence of the TCR-CD3 complex in the rafts before and after engagement is controversial (Montixi et al., 1998; Janes et al., 1999; Kosugi et al., 1999). It appears that the association is weak and sensitive to most non-ionic detergents but is readily detectable by biochemical means using certain polyoxyethylene ether (Brij) detergent series (Montixi et al., 1998; Galbiati et al., 2001) or in situ by immunofluorescence analysis in the absence of detergent (Janes et al., 1999). As a consequence of both raft redistribution and cytoskeletal reorganisation, a supramolecular activation complex (the immunological synapse) containing the assembled signalling machinery is formed at the interface of the T lymphocyte and the antigen-presenting cell (APC) (Monks et al., 1998; Grakoui et al., 1999; Dustin and Chan, 2000). Upon assembly of the signalling machinery, the cytoskeleton is reorganised,



and the Ras/MAPK and PLCγ1 cascades are activated within the rafts, which produce signals that stimulate T cell proliferation (Lin and Weiss, 2000).

The tyrosine kinase activity of Src kinases is essential for T cell activation. This activity is regulated negatively by Csk, a tyrosine kinase that phosphorylates Src kinases at an inhibitory C-terminal tyrosine residue. Csk is recruited into rafts through an interaction between its SH2 domain and the cytoplasmic domain of PAG/Cbp, a transmembrane adapter protein constitutively present in rafts, upon phosphorylation of a specific tyrosine residue present in the cytoplasmic domain (Awabuchi et al., 2000; Brdicka et al., 2000). This phosphorylation is probably mediated by a Src kinase, thus providing an autoregulatory loop for Src kinases. Proline-enriched protein tyrosine phosphatase (PEP), a tyrosine phosphatase associated with Csk that dephosphorylates the activating phosphorylation sites of Lck and Fyn (Cloutier and Veillette, 1999), might be involved in PAG/Cbp dephosphorylation (Torgersen et al., 2001). The CD45 protein tyrosine phosphatase, an integral protein excluded from rafts, can both positively and negatively regulate Lck molecules present at the edge of the rafts by dephosphorylating the C-terminal and autophosphoylation sites, respectively (Rodgers and Rose, 1996). Other possible substrates of CD45 are LAT, CD3ζ and other subunits of the CD3 complex. SH2-domain-containing protein tyrosine phosphatases SHP-1 and SHP-2 might also participate in the dephosphorylation of substrates through recruitment into rafts by interactions with transmembrane adapter proteins (Kosugi et al., 2001; Wei-Chih et al., 2001).

In addition to the TCR, other multichain immune recognition receptors, such as the B cell antigen receptor (BCR) and the high-affinity IgE receptor (FcεRI) of mast cells, appear to use lipid rafts for signalling (Langlet et al., 2000; Cherukuri et al., 2001). Using high-resolution transmission electron microscopic analysis, Wilson and co-workers recently showed that in resting cells FcεRI colocalises loosely with the Src family kinase Lyn in small clusters, whereas LAT occurs in clusters distinct from those containing the receptor (Wilson et al., 2000; Wilson et al., 2001). Upon FcεRI crosslinking, two different processes take place: (1) FcεRI redistributes into specialised domains that exclude Lyn and accumulate the tyrosine kinase Syk, PLCγ2 and a portion of the p85 subunit of PI3-K and other signalling molecules; and (2) LAT clusters rapidly enlarge without mixing extensively with the FcεRI clusters, and LAT associates with PLCγ1 and p85. Biochemical analysis indicated that both FcεRI and LAT are present in rafts in mast cells (Wilson et al., 2001). Therefore, mast cells might propagate activation signals from two distinct types of raft subdomain: primary subdomains organised around FcεRI; and secondary subdomains, including clusters organised around LAT. Whether a similar topographical segregation of signalling subdomains applies also to T and B lymphocytes remains to be established.

Mobilisation of lipid rafts upon T cell costimulation

Unlike triggering by extensive TCR crosslinking with an excess of anti-CD3 antibodies, triggering by APCs involves displaying limited amounts of processed antigen peptides, which cannot produce massive TCR engagement directly. Costimulatory signals provided by other receptor-ligand interactions are necessary for T cell activation under these conditions. CD28 functions as the major T cell costimulatory receptor, but other T cell surface molecules can induce T cell costimulation as well. The nature of these signals has been recently explained in terms of raft reorganisation. Thus, whereas the crosslinking of the TCR alone by suboptimal amounts of anti-CD3 antibodies does not result in clustering of the rafts, coengagement of CD28 redistributes lipid rafts to the contact site (Viola et al., 1999). Reorganisation of the actin cytoskeleton to the contact site is required for the sustained signalling that leads to T cell activation (Valitutti et al., 1995). In addition to promoting translocation of the rafts, costimulation through CD28 induces movement of receptors linked to the actin cytoskeleton to the T-cell–APC interface (Wülfing and Davis, 1999). Similarly, costimulation through the raft-associated GPI-anchored molecule CD48 enhances the recruitment of hyperphosphorylated CD3ζ to the rafts and targets TCR-CD3 elements to the cytoskeleton (Moran and Miceli, 1998). Thus, costimulation through accessory molecules appears to involve a dynamic reorganisation of rafts to surround the TCR molecules, a process requiring simultaneous engagement of the TCR (Yashiro-Ohtani et al., 2000). Although the mechanism of CD28-costimulation-dependent migration of rafts to the contact site has not yet been elucidated, this process is known to be disrupted by the expression of kinase-active/SH3-impaired Lck mutants (Patel et al., 2001) and negatively regulated by the Cbl-b adapter (Krawczyk et al., 2000). The observations that, unlike the TCR present in mature T cells, the pre-TCR of $CD4^-$ $CD8^-$ thymocytes is constitutively present in rafts (Saint-Ruf et al., 2000) and that the coalescence of rafts triggered by TCR and CD28 costimulation takes place in mature T cells but not in inmature $CD4^+$ $CD8^+$ thymocytes (Ebert et al., 2000) indicate that the use of rafts is regulated during T cell differentiation.

The role of rafts in T cell activation

It is increasingly clear that lipid-raft aggregation accompanies signalling following TCR engagement (Janes et al., 1999), that the activation process requires machinery able to access rafts (Kabouridis et al., 1997; Lin et al., 1999) and that raft integrity is necessary for efficient binding of TCR to MHC class I molecules (Drake and Braciale, 2001). The exact role of the rafts in the activation process, however, is still under debate. Treatment of T cells with polyunsaturated fatty acids impairs activation signals (Stülnig et al., 1998), but whether this treatment is specific to the lipid rafts and, if so, how rafts are perturbed is not known. Conflicting effects of methyl-β-cyclodextrin (a cholesterol-sequestering agent) on T cell activation have been reported. Seed and co-workers found that treatment with methyl-β-cyclodextrin impairs activation processes, suggesting that raft integrity is required for T cell activation (Xavier et al., 1998). However, Kabouridis et al. have reported that treatment with methyl-β-cyclodextrin itself induces different activation pathways in T cells, suggesting that rafts are required only to keep apart the activation machinery, which would otherwise form an ensemble without TCR engagement (Kabouridis et al., 2000). Moreover, the coalescence with the TCR-CD3 complex of rafts containing Lck, GM1 and cholesterol, but not LAT, has recently been questioned in studies using immunoisolation of plasma

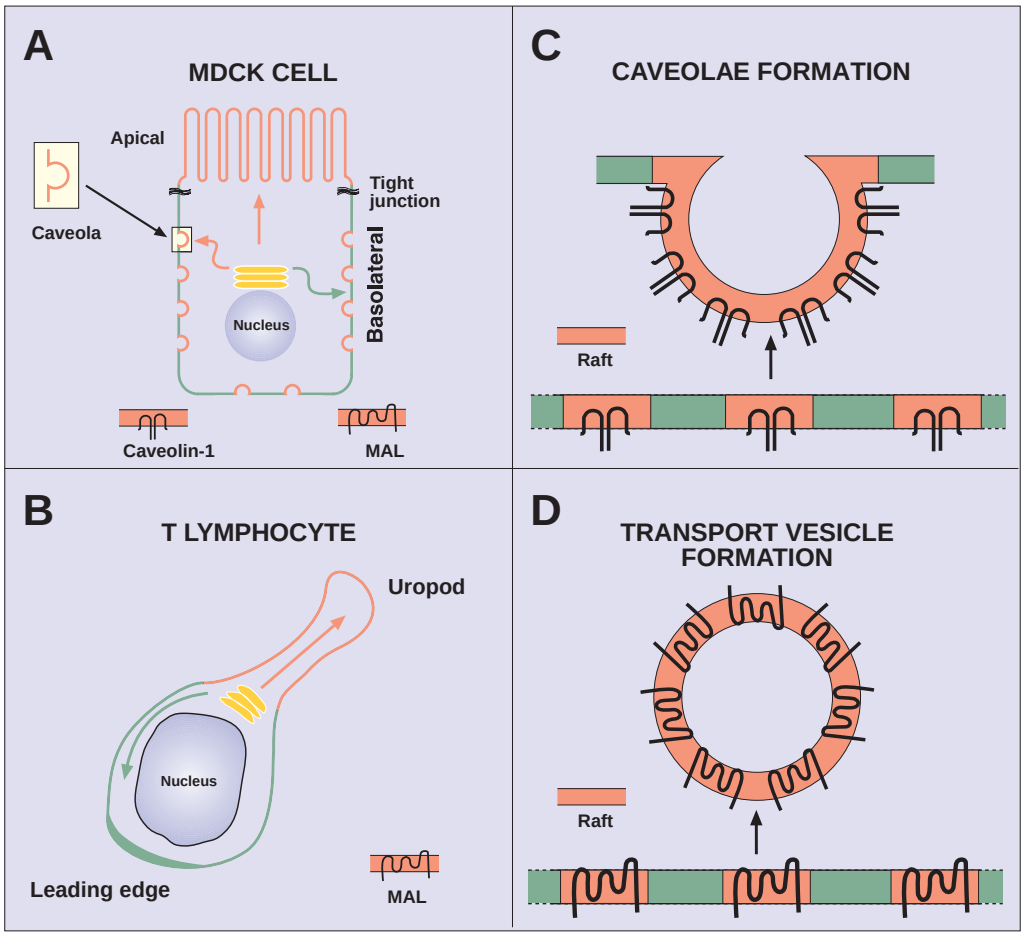


Fig. 3. Transport pathways in polarised epithelial MDCK cells and T lymphocytes. (A) In MDCK epithelial cells, newly synthesised proteins are segregated after passage through the Golgi in different vesicular carriers destined for the apical (red) and basolateral (green) subdomains, which have different protein compositions and functions. Partitioning of proteins into rafts appears to mediate the sorting of at least some apical membrane proteins, such as HA, whereas basolateral sorting (green arrow) is dependent on the existence of a specific signal in the cytoplasmic tail of membrane proteins. Caveolae are raft-containing invaginated structures exclusively located in the basolateral surface. MAL and caveolin-1 are machinery involved in raft-dependent apical transport (straight arrow in red) and caveolae formation (curved arrow in red), respectively. (B) Polarised migrating T lymphocytes display two poles: the leading edge at the front and a membrane protrusion (the uropod) at the trailing edge, each of which has a specific protein composition and function. HA appears to employ rafts for biosynthetic transport (red arrow) to the uropod, which contains rafts. T cells lack caveolin-1 but do express MAL. (C) Caveolin-1 is necessary for caveolae formation and organises lipid rafts to build the caveolar architecture. (D) MAL is necessary for apical transport and appears to organise lipid rafts for the formation of the transport vesicles.

membrane subdomains containing TCR-CD3 complexes prepared in the absence of detergent (Harder and Kuhn, 2000).

Rafts in membrane trafficking in epithelial MDCK cells and T lymphocytes

The existence of rafts was originally postulated to explain the specific sorting of glycolipids and proteins to the cell surface of polarised MDCK epithelial cells (Simons and Wandinger-Ness, 1990) (Fig. 3A). Their model was mainly based on the following findings: (1) the preferential targeting of both influenza virus hemagglutinin (HA) and glycolipids to the apical surface in polarised epithelial MDCK cells; (2) the insolubility of glycolipid-enriched membranes in non-ionic detergents at low temperatures; and (3) the fact that newly synthesised HA becomes insoluble during biosynthetic transport to the cell surface. The last observation was interpreted as meaning that HA associates in the Golgi with glycolipid and cholesterol-containing vesicular carriers destined for the apical surface. This model drew experimental support from the observation that transport of HA to the apical surface is impaired by the disruption of raft integrity by cholesterol sequestration (Keller and Simons, 1998). In T cells, a polarised morphology is evident when the cells carry out certain functions, such as migration or cell-cell interactions (Sánchez-Madrid and del Pozo, 1999). The poles of a migrating T cell display specific features related to the specialised function of these cells in the immune response. Thus, in addition to its general role in adhesion to the substrate during migration, the leading edge in T lymphocytes constitutes a zone of high sensitivity to antigen and chemotactic cytokines (Negulescu et al., 1996; Nieto et al., 1997). The trailing end forms a characteristic membrane protrusion, the uropod, that selectively concentrates molecules involved in intercellular adhesion, such as ICAM-1, ICAM-2 and ICAM-3, CD43 and CD44 (Sánchez-Madrid and del Pozo, 1999) (Fig. 3B). In common with targeting of HA to the apical surface, HA becomes integrated into rafts soon after biosynthesis (Millán et al., 2002) and is selectively sorted to the uropod protrusion (Fig. 4). Thus, T lymphocytes appear to have a transport route reminiscent of that of the apical pathway in MDCK cells, which could target specific proteins to the uropod. Note that all surface HA is detected in raft lipids (Millán et al., 2002), indicating that the uropod tip is rich in rafts. The selective targeting of HA suggests that the uropod rafts have a lipid composition different from those containing TCR-signalling-sensitive molecules at the leading edge. The raft-mediated pathway of transport to the uropod might be

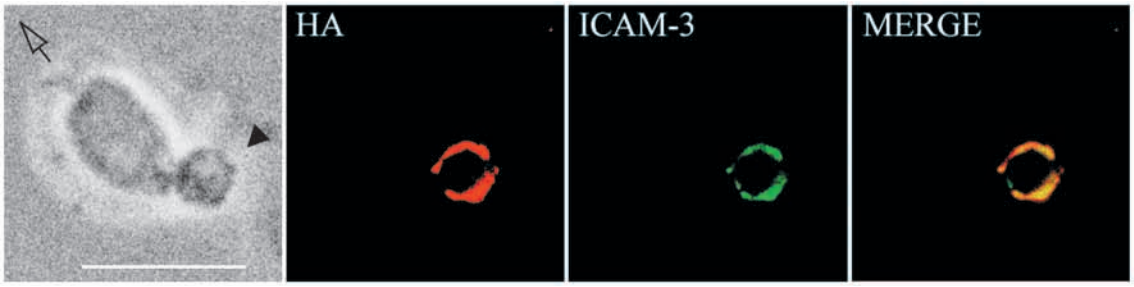


Fig. 4. Vectorial transport of HA in T lymphocytes. Polarised T lymphoblasts infected with the influenza virus were fixed and subjected to double-label immunofluorescence analysis with antibodies specific to HA and to ICAM-3, a uropod protein marker, in the absence of a permeabilization step. The bright field image is depicted to show the cell morphology. The uropod and the direction of migration are indicated by an arrowhead and an arrow, respectively. Controls to assess the specificity of the labelling included incubations with control primary antibodies or omission of the primary antibodies. Bar, 5 μm.

involved in the generation of a raft reservoir at the T cell surface, which could subsequently be used for the formation of the immunological synapse and in the specific delivery of intracellular raft proteins and lipids to this site after TCR engagement. In support of this view, TCR triggering induces transport of the GM1 ganglioside and Lck from an intracellular store to the plasma membrane (Tuosto et al., 2001) and translocation of Lck-associated protein kinase C-θ to rafts (Bi et al., 2001), which localise to the T cell synapse. These findings imply that there is a link between the exocytic/endocytic trafficking of lipids and proteins and T cell signalling.

In addition to the role of the rafts in exocytic transport, an endocytic pathway involving rafts mediates the internalisation of GPI-anchored proteins and interleukin 2 receptors by a clathrin-independent mechanism (Bamezai et al., 1992; Deckert et al., 1996; Lamaze et al., 2001). Therefore, it is conceivable that, in addition to laterally diffusing along the plasma membrane, raft proteins and lipids are internalised and transported to the contact site to build the immunological synapse.

Lessons from other cell types: specific protein machinery for raft-mediated processes

In epithelial cells and fibroblasts, raft reorganisation for transport or signalling processes involves specialised protein machinery that recruits and structures the appropriate rafts. Raft-containing vesicular invaginations of the plasma membrane known as caveolae are involved in signalling and clathrin-independent endocytosis in epithelial cells and fibroblasts (Anderson, 1998). In polarised epithelial cells, caveolae are restricted to the basolateral surface (Scheiffele et al., 1998) (Fig. 3A). Caveolin-1 is a multifunctional raft-associated protein primarily identified as a component of the caveolar architecture (Smart et al., 1999; Razani et al., 2000). Caveolin-1 and another member of the caveolin family, caveolin-2, are involved in the biogenesis of caveolae, which probably involves a raft-mediated pathway from the trans-Golgi network to the basolateral membrane (Scheiffele et al., 1998). Caveolin-1 directs the organisation of rafts into caveolae-like vesicles (Fra et al., 1995) (Fig. 3C) and forms a scaffold onto which many classes of signalling molecule are recruited to generate pre-assembled signalling complexes within caveolae. Signal transducing proteins known to interact with caveolin-1 include Src family kinases, Ras, eNOS, PKCα, PKA, MEK/ERK and heterotrimeric G proteins (Smart et al., 1999). Upon ligand binding, a number of membrane receptors migrate to the caveolae to exploit the pre-assembled signalling machinery stored in these structures. The existence of a family of raft-associated proteins similar to caveolin-1, the caveolin family, suggests that caveolins are elements of the machinery involved in raft organisation (Razani et al., 2000).

MAL is an integral membrane proteolipid protein that selectively resides in lipid rafts in polarised epithelial cells (Zacchetti et al., 1995; Martín-Belmonte et al., 1998). An essential role for MAL in apical sorting has recently been demonstrated: depletion of endogenous MAL severely reduces transport of HA and GPI-anchored proteins to the apical surface in epithelial MDCK cells (Cheong et al., 1999; Puertollano et al., 1999; Martín-Belmonte et al., 2000). MAL continuously cycles from the Golgi to the plasma membrane and endosomes (Puertollano and Alonso, 1999). Consensus sorting motifs in the MAL C-terminus appear to regulate the shuttling of the vesicles and, hence, cargo transport (Puertollano et al., 2001). These findings, together with the observation that overexpression of MAL is able to direct the de novo formation of vesicles (Puertollano et al., 1997), are interpreted as signifying that MAL organises internal rafts for formation of the apical transport carriers (Puertollano et al., 2001) (Fig. 3D). The central role of MAL in apical transport, the existence of a family of proteins that have significant overall sequence identity with MAL (Pérez et al., 1997) and the observation that a new member of this family, BENE, is present in lipid rafts in endothelial-like ECV304 cells (de Marco et al., 2001) are all consistent with the idea that the MAL family of proteins constitutes machinery for raft organisation.

Specific machinery for raft organisation in T cells?

Extensive aggregation of GPI-anchored proteins is not able to induce full activation signals in T cells, which indicates that clustering of surface rafts alone might not be sufficient to elicit the activation process (Moran et al., 1998; Millán et al., 2001). Therefore, it appears that rafts need to be reorganised in specific ways for activation. The LAT adapter (Wilson et al., 2001) and members of the flotillin/reggie protein family (Stuermer et al., 2001; Volonté et al., 1999) are candidates for elements of the machinery involved in raft remodelling in T

lymphocytes (Galbiati et al., 2001). Although T cells are able to assemble signalling- and transport-competent rafts, structures that have a morphology characteristic of caveolae are conspicuously absent in these cells (Fra et al., 1994). Moreover, they do not express caveolin-1, and no expression of members of the caveolin family has yet been described in T cells. Thus, in contrast to other cell types, T cells do not use caveolins to organise rafts.

Despite its restricted range of tissue expression, the *MAL* gene is expressed in T lymphocytes and polarised epithelial cells (Martín-Belmonte et al., 1998). In both cell types, MAL resides in lipid rafts located in the perinuclear region and on the plasma membrane (Zacchetti et al., 1995; Martín-Belmonte et al., 1998; Millán et al., 1997). Given the demonstrated role of MAL in raft-mediated transport in MDCK cells, one obvious hypothesis is that MAL is involved in raft-mediated trafficking in T lymphocytes. Indeed, we have identified MAL, together with Lck, in rafts isolated from an endosomal fraction in the Jurkat T cell line (Millán and Alonso, 1998). MAL might thus be involved in translocating Lck and raft lipids to the immunological synapse upon TCR engagement.

Conclusion and perspectives

Recent research has yielded a plethora of information on the role of lipid rafts in T cell activation. Most of the work done on T cells has developed in parallel to and independently of that in other cell systems. The identification of the protein machinery involved in the organisation of rafts in epithelial cells and fibroblasts has been an important contribution to the field. However, the role in T cells of similar or novel machinery that organises lipid rafts has scarcely been investigated. Now might be the time to examine in T cells which type of rafts intervene in the activation process, how rafts are structured and stabilised, what role is played by the exocytic and endocytic pathways in surface raft reorganisation, what machinery is involved, and whether or not there is a structural link between the machinery involved in the organisation of lipid rafts in T lymphocytes and that used in other cell types.

We thank P. Aganzo for his help with the artwork, and B. Alarcón for critically reading the manuscript. J.M. is the recipient of a postdoctoral fellowship from the Comunidad de Madrid. This work was supported by grants from the Ministerio de Ciencia y Tecnología (PM99-0092), the Comunidad de Madrid (08.3/0025/2000) and the Fondo de Investigación Sanitaria (01/0085-01).

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