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Lipid rafts in immune signalling: current progress and future perspective

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Summary

Lipid rafts are dynamic assemblies of proteins and lipids that harbour many receptors and regulatory molecules and so act as a platform for signal transduction. They float freely within the liquid-disordered bilayer of cellular membranes and can cluster to form larger ordered domains. Alterations in lipid rafts are commonly found to be associated with the pathogenesis of several human diseases and recent reports have shown that the raft domains can also be perturbed by targeting raft proteins through microRNAs. Over the last few years, the importance of lipid rafts in modulating both innate and acquired immune responses has been elucidated. Various receptors present on immune cells like B cells, T cells, basophils and mast cells associate with lipid rafts on ligand binding and initiate signalling cascades leading to inflammation. Furthermore, disrupting lipid raft integrity alters lipopolysaccharide-induced cytokine secretion, IgE signalling, and B-cell and T-cell activation. The objective of this review is to summarize the recent progress in understanding the role of lipid rafts in the modulation of immune signalling and its related therapeutic potential for autoimmune diseases and inflammatory disorders.

Keywords: autoimmune disease; B/T-cell activation; cytokine signalling; IgE; lipid rafts; microRNA; Toll-like receptor.

Introduction

The plasma membrane is an important component of the cell and a large number of studies have been conducted into understanding the membrane structure and function. In 1972, Singer and Nicolson proposed the 'Fluid mosaic model' of biological membranes, which describes it as 'protein icebergs floating in the sea of lipid'.[1] In the early 1980s, a series of experimental findings revealed that the lipids are not uniformly distributed in the cell membrane, and the concept of membrane microdomain 'lipid rafts' was postulated.[2,3] Figure 1 depicts the timeline of pioneer studies in the lipid raft field.

Lipid rafts are defined as small (10–200 nm) heterogeneous, highly dynamic, sterol (cholesterol), sphingolipid- and protein-enriched domains that compartmentalize the cellular processes.[4] They incorporate several distinct classes of proteins – true resident proteins (glycosylphosphatidylinositol-linked proteins, caveolin, flotillin), signalling proteins (doubly acylated proteins like Src family kinases), G-protein-coupled receptor (GPCR) proteins, cholesterol-linked and palmitoylated proteins such as hedgehog and myristoylated proteins.[5] Although the existence of lipid rafts remains controversial, there are several theories that explain its formation in biological membranes. According to one theory, self-associative properties of sphingolipid and cholesterol facilitate selective

Abbreviations: BCR, B-cell receptor; DRMs, detergent-resistant membrane fractions; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; IFN, interferon; IL-8, interleukin-8; ITAM motif, immunoreceptor tyrosine-based activation; LAT, linker of activation of T cells; LBP, LPS-binding protein; Lck, lymphocyte-specific protein tyrosine kinase; LIF, leukaemia inhbitory factor; LIFR, leukaemia inhbitory factor receptor; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MBCD, methyl-β cyclodextrin; NF-κB, nuclear factor-κB; SLE, systemic lupus erythematosus; TCR, T-cell receptor; TGF, transforming growth factor; TLR, Toll-like receptor; TIR domain, TLR/interleukin-1 receptor homology domain; TNF, tumour necrosis factor; TRAF6, TNF receptor-associated factor 6

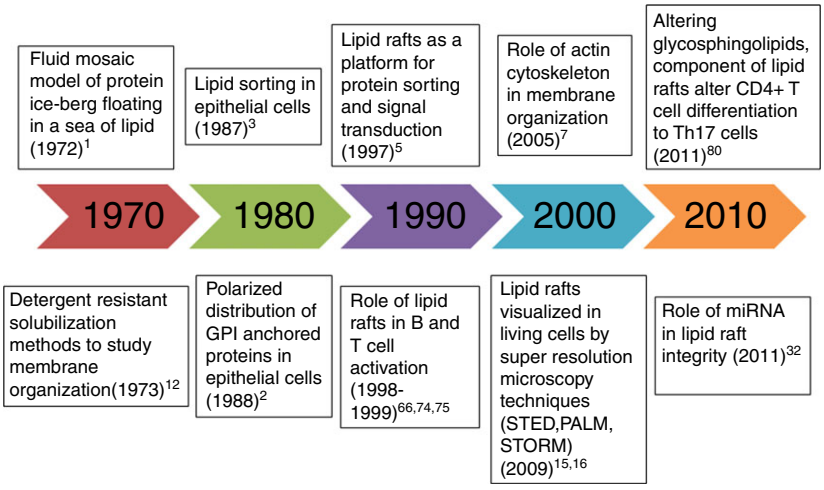

Figure 1. Timeline of pioneer discoveries in the field of lipid rafts and immune signalling.

lateral segregation in the membrane plane. Another hypothesis suggests that rafts are constructed of lipid shells, which are 1–10 nm thermodynamically stable mobile entities of proteins preferentially associated with certain types of lipids (protein–lipid shell). Lipid shells target the proteins they encase to pre-existing raft/caveolar domains and they could be the quantal unit of rafts.[6] Further, Kusumi *et al.*[7] proposed the 'picket–fence model', which explains that the anchoring of transmembrane proteins to the underlying actin cytoskeleton restricts lateral diffusion of proteins and is therefore involved in compartmentalization of the membrane. The evidence suggests that lipid raft formation is driven and stabilized by lipid–lipid, lipid–protein and protein–protein interactions.

There are generally two types of lipid rafts: planar lipid rafts (also known as non-caveolar) and caveolae. Planar rafts are continuous non-invaginated membrane domains that lack distinguishing morphological features whereas caveolae are flask-shaped invaginated membrane structures formed by polymerization of caveolin proteins. Caveolins are transmembrane palmitoylated proteins having a hairpin-like structure that binds tightly to cholesterol.[8] These are present in three isoforms (caveolin-1, -2 and -3) and are transcribed from different genes. Out of the three caveolins, caveolin-1 is known to modulate inflammatory responses. Medina *et al.*[9] found that knockdown of the caveolin-1 protein leads to defects in innate immunity and mice become more susceptible to infection. Caveolin-2 is involved in lung functions while caveolin-3 is a muscle-specific isoform and its knockdown in mice results in muscular dystrophy symptoms.[10]

Two widely used methods to study lipid rafts are the isolation of detergent-resistant membrane fractions (DRMs) and cholesterol depletion.[11,12] DRM analysis is not considered reliable because DRM fractions vary in lipid and protein composition depending upon the type of detergent, its concentration, temperature conditions and different cell types.[13] Cholesterol depletion by methyl-β cyclodextrin (MBCD) suffers from serious adverse effects; in addition to raft disruption, it also perturbs many other cellular functions.[14] Due to the lack of proper methodologies for studying rafts, several questions about its existence have been raised. Later in 2000, advanced microscopy techniques such as fluorescence resonance energy transfer, fluorescence polarization anisotropy and single-particle tracking, fluorescence correlation spectroscopy, supported the existence of dynamic cholesterol-dependent nano-clusters in the cell membrane. Furthermore, super-resolution microscopy techniques including stimulated emission depletion, photoactivated localization microscopy and stochastic optical reconstruction microscopy that provide resolution substantially below the diffraction limit, demonstrated the presence of raft domains in the cell membrane.[15,16]

MicroRNAs as modulators of lipid rafts in human diseases

Lipid rafts play a crucial role in protein sorting and receptor-mediated signal transduction, providing a distinct environment for the functioning of receptors and intracellular molecules. These nanodomains have been known to be altered in various diseases such as neurological disorders, cancer, cardiovascular diseases, insulin resistance, microbial infections and inflammatory diseases. As shown in Fig. 2,[17–29] various molecules involved in disease progression have also been found to be associated with lipid rafts and caveolae.

Recently, several reports have suggested that microRNAs act as critical regulators of lipid raft structure by targeting raft-associated proteins (caveolin and flotillin). MicroRNAs are single-stranded non-coding RNAs (~ 22 nucleotides in length) that are ubiquitously present in plants and animals and act in a sequence-specific manner to regulate gene expression at the post-transcriptional

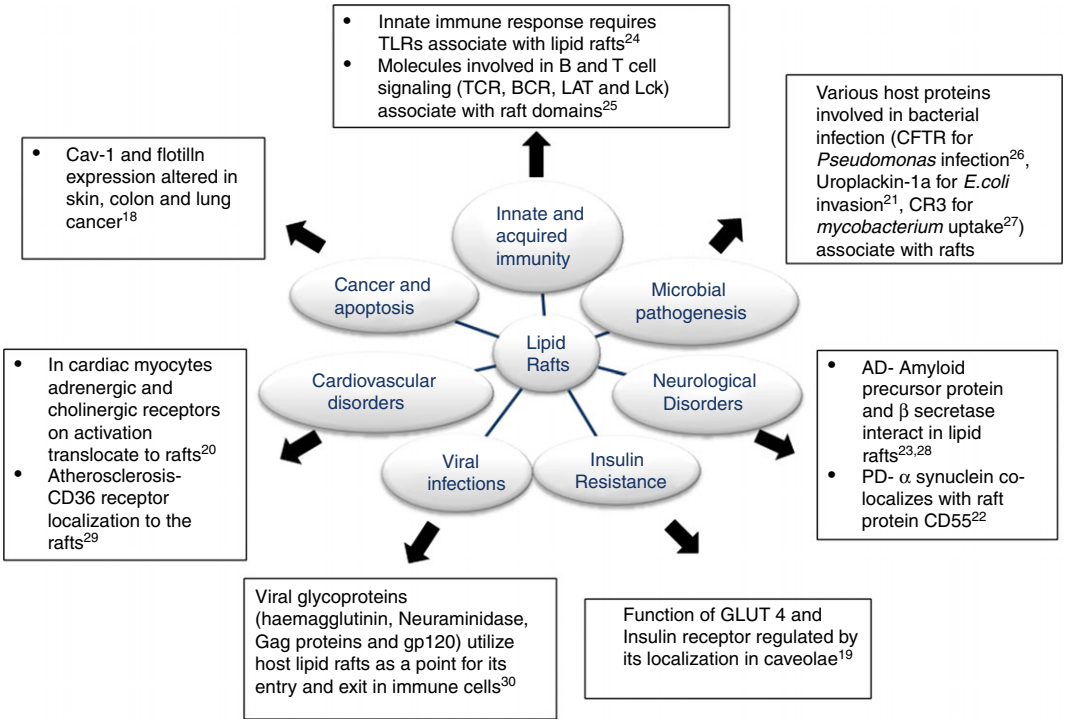

Figure 2. The role of lipid rafts in health and disease – Interaction of various molecules known to be involved in the pathogenesis of various diseases with lipid rafts. Abbreviations: AD, Alzheimer disease; PD, Parkinson's disease; CFTR, cystic fibrosis transmembrane conductance regulator; CR3, complement receptor 3; GLUT4, glucose transporter.

level by cleavage or translational repression of their target mRNAs.[30] Findings over the past few years have strongly supported the role of microRNAs in the regulation of crucial cellular processes such as cell proliferation, apoptosis, adipocyte and myoblast differentiation, fat metabolism, neuronal paternity, developmental regulation and cell differentiation in mammals.[31] The first report on the role of microRNA in regulating lipid raft components was published in 2011 when Nohata *et al.*[32] showed that miR-133a attenuates metastasis in head and neck squamous cell carcinoma cells by targeting caveolin-1. Other studies from our laboratory and other groups showed that microRNAs (miR-195, miR-203, miR-199a-3p and miR-218) significantly inhibit migration and invasion in cancer cells by targeting caveolins.[33–37] In an independent study, Li *et al.*[38] found the anti-metastatic activity of miR-124 against breast cancer cells to be flotillin-dependent. Later, Gong *et al.*[39] found that miR-138 negatively regulates nuclear factor-κB (NF-κB) activation in oesophageal cancer cells by perturbing assimilation of NF-κB signalling intermediates [TNF receptor-associated factor 2 (TRAF2), receptor-interacting protein kinase-1 (RIP1)] within the lipid raft domain by targeting caveolin-1, flotillin-1 and flotillin-2. The microRNA miR-124 was also found to regulate caveolae-mediated endocytosis of pathogens as well as acrosome biogenesis during spermatogenesis by specifically targeting flotillin-2.[40,41]

Apart from cancer pathogenesis, the involvement of miRNAs in mediating regulation of lipid raft components has also been implicated in the pathogenesis of several other diseases (Table 1). Trajkovski *et al.*[42] in their study found that miR-103/107 regulates glucose homeostasis and insulin sensitivity in obese mice by modulating the levels of caveolin-1. Furthermore, loss of function by antagonizing miR-103/107 levels leads to the up-regulation of caveolin-1, which in turn stabilizes the insulin receptor and enhanced insulin signalling. In an independent study, Hoeke *et al.*[43] observed that miR-29a regulates cellular uptake of pathogens during intestinal salmonella infection by way of caveolin-2-mediated targeting of focal adhesion and the actin cytoskeleton pathway. Similar to these, Chen *et al.*[44] and Lino Cardenas *et al.*[45] showed that miR-22 and miR-199-5p possess caveolin-mediated regulatory and protective effects during cardiac and lung injury, respectively. Recently, Sang *et al.*[46] reported the inhibitory action of miR-150 against inflammatory cytokine production. miR-150 induces immune tolerance not only by decreasing the levels of ARRB2/AKT/PDE4 (arrestin beta 2/ protein kinase B/ phosphodiesterase 4) but also by preventing their recruitment inside the lipid raft domain. As lipid rafts have been known to be crucial players in innate and acquired immunity,[24] we herein review the involvement of these membrane domains in various immune

Table 1. List of microRNAs altering disease physiology by targeting raft proteins (caveolin and flotillin)

miRNA	Target	Activity	References
miR-133a	Caveolin-1	Regulates migration and invasion in head and neck squamous cell carcinoma	32
miR-124	Caveolin-1	Reduces caveolar density in porcine kidney cells and regulates caveolae-mediated endocytosis of pathogens	41
	Flotillin-1	Regulates migration and invasion in breast cancer cells	38
	Flotillin-2	Regulates mouse acrosome biogenesis during spermatogenesis	40
miR-203	Caveolin-1	Regulates migration and invasion in pancreatic cancer cells	33
		Regulates longevity and aging in breast tissue during caloric restriction	37
miR-138	Flotillin-1, -2, Caveolin-1	Regulates nuclear factor-κB signalling pathway involved in aggressiveness of oesophageal squamous cell carcinoma	39
miR-103/107	Caveolin-1	Regulates insulin sensitivity in obese mice	42
miR-199-5p	Caveolin-1	Regulator of tissue fibrosis during lung fibrogenesis	45
miR-199a-3p	Caveolin-2	Regulates proliferation and survival of endothelial and breast cancer cells	34
miR-218	Caveolin-2	Regulates migration and invasion in renal cell carcinoma	36
miR-29a	Caveolin-2	Inhibits endocytosis of pathogens during salmonella infection	43
miR-22	Caveolin-3	Regulates endothelial nitric oxide synthase activity during cardiac injury	44
miR-150	–	Inhibits nuclear factor-κB signalling by perturbing the recruitment of AKT/ARRB2/PDE4 into the lipid raft	46

signalling pathways with their mechanisms in detail. We also describe how alterations in lipid rafts can be used as therapeutics for immunological disorders.

Lipid rafts involved in pathogen recognition via Toll-like receptor signalling

Toll-like receptors (TLRs) are part of the innate immune system that act as the primary sensors of pathogens, and the literature reveals that the integrity of lipid rafts is crucial for normal TLR signalling. There are 10 members of the TLR family, some of which are present on the cell surface (TLR1, TLR2, TLR4, TLR5 and TLR6) whereas others are present intracellularly (TLR3, TLR7, TLR8 and TLR9). Diverse TLRs recognize diverse pathogen-associated molecular patterns and trigger secretion of pro-inflammatory cytokines. Binding of the ligand recruits adaptor toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) through the TIR domain (TLR/interleukin-1 receptor homology domain) to initiate downstream signalling. TIRAP recruits MyD88, interleukin-1 receptor-associated kinase and TRAF6 to the receptor complex and activates transforming growth factor-β-activated kinase, which then further activates transcriptional factors like NF-κB and interferon regulatory factors resulting in the production of the cytokines tumour necrosis factor-α (TNF-α), interleukin 8 (IL-8), IL-6 etc.

The innate immune response towards bacterial lipopolysaccharide (LPS) is facilitated by its binding with LPS-binding protein (LBP). The resulting LPS–LBP complex then binds to CD14, a lipid raft-associated glycosylphosphatidylinositol-anchored protein, which then transduces signals by associating with TLR4 and recruiting downstream signalling molecules as shown in Fig. 3(a).[47] Microscopy studies (fluorescence resonance energy transfer and fluorescence recovery after photobleaching) have shown that LPS induces the clustering of TLR4 in lipid rafts. In addition to CD14, several other raft proteins such as CD36, CD44, heat-shock protein 90 are also known to be involved in recognition of LPS and non-microbial products that participate in TLR4 signalling.[48,49] The intracellular juxtamembrane domains of several Toll-like receptors are known to possess Cholesterol Recognition Amino-Acid Consensus sequences, which suggests a direct role of cholesterol in the activation process.[50] Polyunsaturated fatty acids are known to inhibit TLR signalling by inhibiting receptor dimerization and TLR recruitment to the lipid rafts.[51] Furthermore, it has been observed that increasing cellular cholesterol increases the number of lipid rafts, which in turn affects TLR signalling. Cholesterol-loading by the cyclodextrin–cholesterol complex in the plasma membrane activates TLR4 signalling whereas in the endosomal membrane, the complex activates TLR3 signalling in macrophages. Zhu *et al.*[52] in their recent study found that cholesterol transporter ATP-binding cassette transporter A1 knockout macrophages showed MyD88-dependent enhanced NF-κB activation and enhanced secretion of pro-inflammatory cytokines in response to TLR2, TLR4, TLR7, TLR9

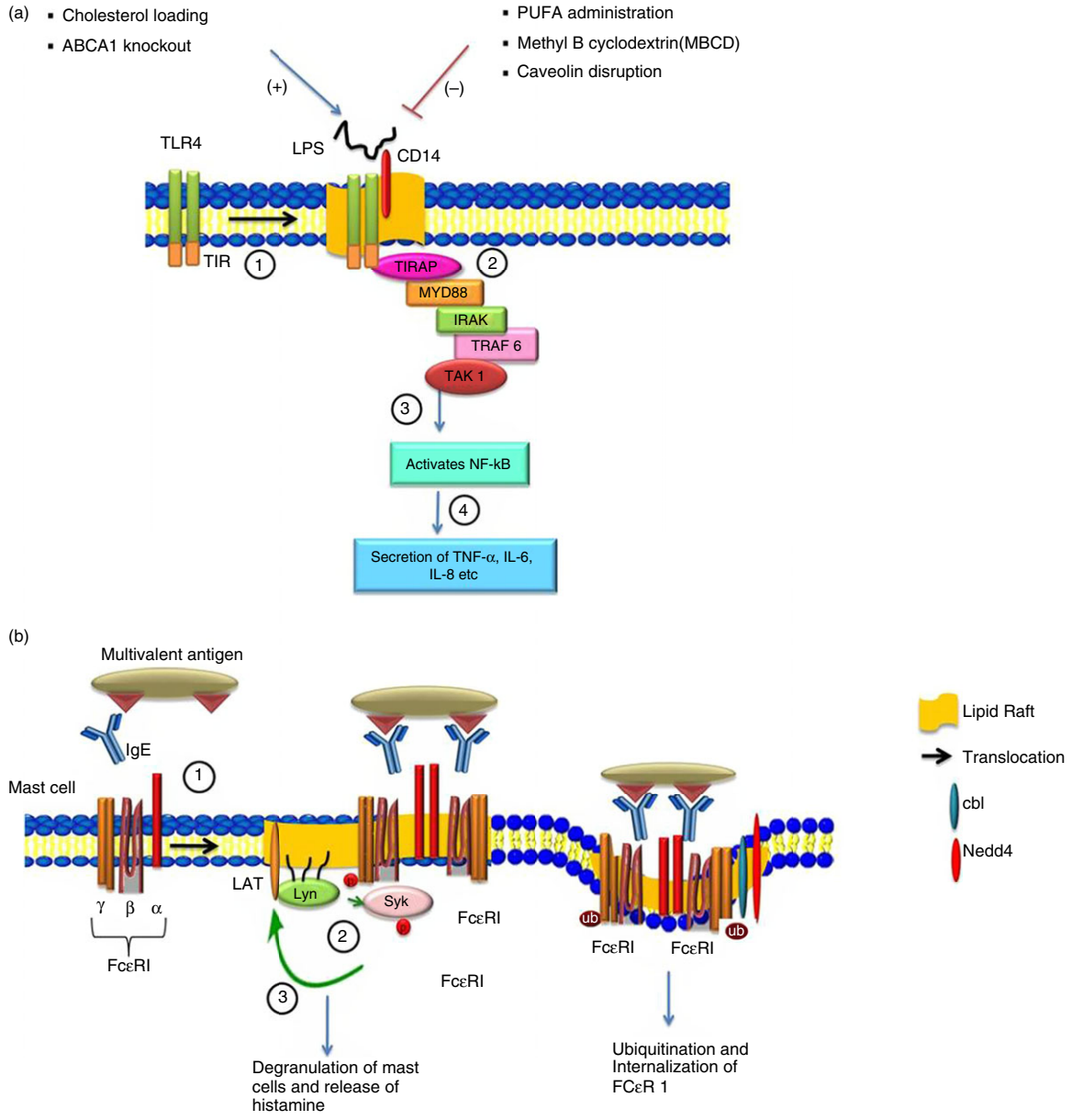

Figure 3. (a) The role of lipid rafts in Toll-like receptor (TLR) signaling. (1) Binding of ligand such as lipopolysaccharide (LPS) with TLR4 results in its translocation to lipid rafts where it interacts with CD14. (2) In lipid rafts, the interaction of toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) with TLR4–CD14 complex through the TLR/interleukin-1 receptor (TIR) domain recruits several adaptor molecules such as MYD88, interleukin-1R-associated kinase (IRAK) and tumour necrosis factor receptor-associated factor 6 (TRAF6) and activates transforming growth factor-β-activated kinase 1 (TAK1). (3) TAK1 further activates nuclear factor-κB (NF-κB) and leads to the secretion of various cytokines like interleukin-6 (IL-6), IL-8 etc. (4) Disruption of lipid rafts by polyunsaturated fatty acid (PUFA) administration and methyl-β cyclodextrin (MBCD) or by caveolin disruption inhibits TLR signalling (−), while increasing the cellular cholesterol and ATP-binding cassette transporter A1 (ABCA1) knockout enhances TLR signalling (+). (b) The role of lipid rafts in IgE signalling. (1) In mast cells, cross-linking of IgE-bound antigen with FcεRI leads to its translocation to lipid rafts. (2) Within the raft domain, a doubly acylated Src-like tyrosine kinase protein Lyn phosphorylates the immunoreceptor tyrosine-based activation (ITAM) domain and results in recruitment and phosphorylation of Syk kinase. (3) Syk further activates linker of activation of T cells (LAT) and recruits several other adaptor molecules in the lipid rafts resulting in the release of chemical mediators such as histamine. (4) Co-localization of ubiquitin ligase Cbl and Nedd4 with FcεRI inside the lipid raft domain results in ubiquitination and internalization of the receptor.

agonists compared with wild-type macrophages. This hyper-responsiveness is due to increased lipid raft-free cholesterol and increased TLR localization to lipid rafts.

Raft protein caveolin-1 is known to regulate TLR signalling by modulating endothelial nitric oxide synthase (eNOS) activity. The eNOS-derived nitric oxide (NO)

blocks platelet and neutrophil activation and inhibits mast cell-induced inflammation. Stable binding of eNOS with caveolin-1 protein suppresses its activity but in the presence of agonist, eNOS becomes dissociated from caveolin-1 and synthesizes NO. Mirza *et al.*[53] reported that the lungs of caveolin-1 knockout mice show reduced TLR4 signalling through sustained eNOS activation and decreased NF-κB activation in response to LPS compared with the lungs of wild-type mice. Pulmonary hypertension, cardiomyopathy and resistance to LPS-induced acute lung injury was observed in cav$^{-/-}$ mice, which suggests an inflammatory effect of caveolae.[54] Similarly, Tsai *et al.*[55] broadly showed that caveolin-1 not only regulates TLR4 signalling but also regulates CD14, CD36 and MyD88 protein expression in macrophages and their response towards bacterial infection. All these studies suggest that recruitment of a TLR complex into lipid rafts and the expression of cav-1 are crucial for modulating the innate immune response.

Lipid rafts in IgE signalling

IgE signalling was the first form of signalling that highlighted the involvement of lipid rafts during allergic and parasitic immune responses. This signalling is initiated when IgE binds to the Fc region of its specific receptor (FcεRI) which is constitutively expressed on mast cells and basophils. FcεRI is a tetrameric molecule that includes one α, one β and two γ chains, the α-subunit binds to the Fc region of IgE and the β and γ subunits have immunoreceptor tyrosine-based activation (ITAM) motifs in their cytoplasmic domain. Cross-linking of these receptors with IgE-bound antigen increases their association with the lipid rafts where Lyn, a doubly acylated Src-like tyrosine kinase protein phosphorylates the ITAM domain of FcεRI and creates a novel binding site recognized by cytoplasmic Syk/Zap70 family tyrosine kinases through its tandem Src homology-2 (SH2) domain.[56] Lyn further phosphorylates Syk and activates linker of activation of T cells (LAT), a palmitoylated raft-associated adaptor molecule that further recruits various molecules in the raft and leads to the degranulation of mast cells (Fig. 3b).[57]

Lipid raft-associated adaptor proteins LAT and non-T cell activation linker (NTAL) act as the positive and negative regulators of FcεRI signalling. In the absence of LAT alone, mast cells showed a slight decrease in secretion of cytokines whereas knockdown of NTAL alone enhances the activity of LAT. Knockdown of both LAT and NTAL completely blocks FcεRI signalling.[58] SHP1/SHP2 (Src homology 2 domain tyrosine phosphatase) phosphatase and protein tyrosine phosphatase ε are two phosphatases that negatively regulate LAT/NTAL and are excluded from lipid rafts during antigen-activated IgE signalling. Exclusion of phosphatases from raft domains provides an environment for the efficient activity of kinases. Young *et al.*[59] found that co-expression of phosphatases in lipid rafts inhibits Lyn kinase activity along with inhibition of FcεRI phosphorylation. Similarly, Sheets *et al.*[60] observed that depletion of cholesterol by MBCD leads to loss of cross-linking between FcεRI and Lyn kinase in mast cells, which subsequently attenuates IgE signalling. Another key constituent of lipid raft, flotillin-1, was identified as an important component of FcεRI-mediated mast cell activation.[61] Ubiquitination of FcεRI regulates the strength of IgE signalling. Mono-ubiquitination of the receptor functions as a signal for endocytosis inside the lipid rafts whereas polyubiquitination leads to proteasomal degradation.[62] Phosphorylation-dependent ubiquitination of FcεRI occurs in lipid rafts, which in turn leads to endocytosis of the cross-linked FcεRI receptor. It regulates the half-life of a receptor on the cell membrane and is therefore involved in regulating the duration of IgE signalling in lipid rafts. Lafont and Simons[63] demonstrated co-localization of ubiquitin ligase Cbl and Nedd4 with FcεRI in lipid rafts where Cbl was shown to mediate FcεRI ubiquitination and Nedd4 to ubiquitinate membrane proteins. Using cyclodextrin (for cholesterol depletion), Molfetta *et al.*[64] showed that lipid raft integrity is essential for receptor ubiquitination and endocytosis, which later participates in downstream signalling.

Role of lipid rafts in lymphocyte activation

Immune cell activation is required for defence against microbes and several reports suggest a crucial role of lipid rafts in their activation. The B-cell receptor (BCR) present on the surface of B cells comprises a ligand-binding moiety (membrane-bound immunoglobulin molecule which binds to antigen) and a signal transduction moiety (two disulphide-linked heterodimer polypeptides Igα/Igβ each of which contains an ITAM). In the resting state, the BCR has little affinity for rafts and so are present in the non-raft domain of the plasma membrane. Binding of antigen to BCR brings conformational change in the receptor and results in the translocation of BCR to rafts.[65] In rafts, the Src family kinase Lyn phosphorylates the Igα/Igβ heterodimer and recruits the cytoplasmic protein kinase Syk, which further initiates the signalling cascade.[66] In immature and anergic B cells, binding of antigen to BCR fails to instigate its association with rafts and further internalization, which in turn triggers apoptosis of the cell. These observations suggest that the association of BCR with rafts is crucial for B-cell activation.[67]

As shown in Fig. 4(a), the B-cell response is augmented or attenuated by the co-receptors present on its surface. Complement receptor-2 (CR2, also known as CD21) is a positive co-receptor in conjunction with two other transmembrane proteins CD19 and CD81 and augments B-cell activation. Similar to BCR, these co-receptors reside in

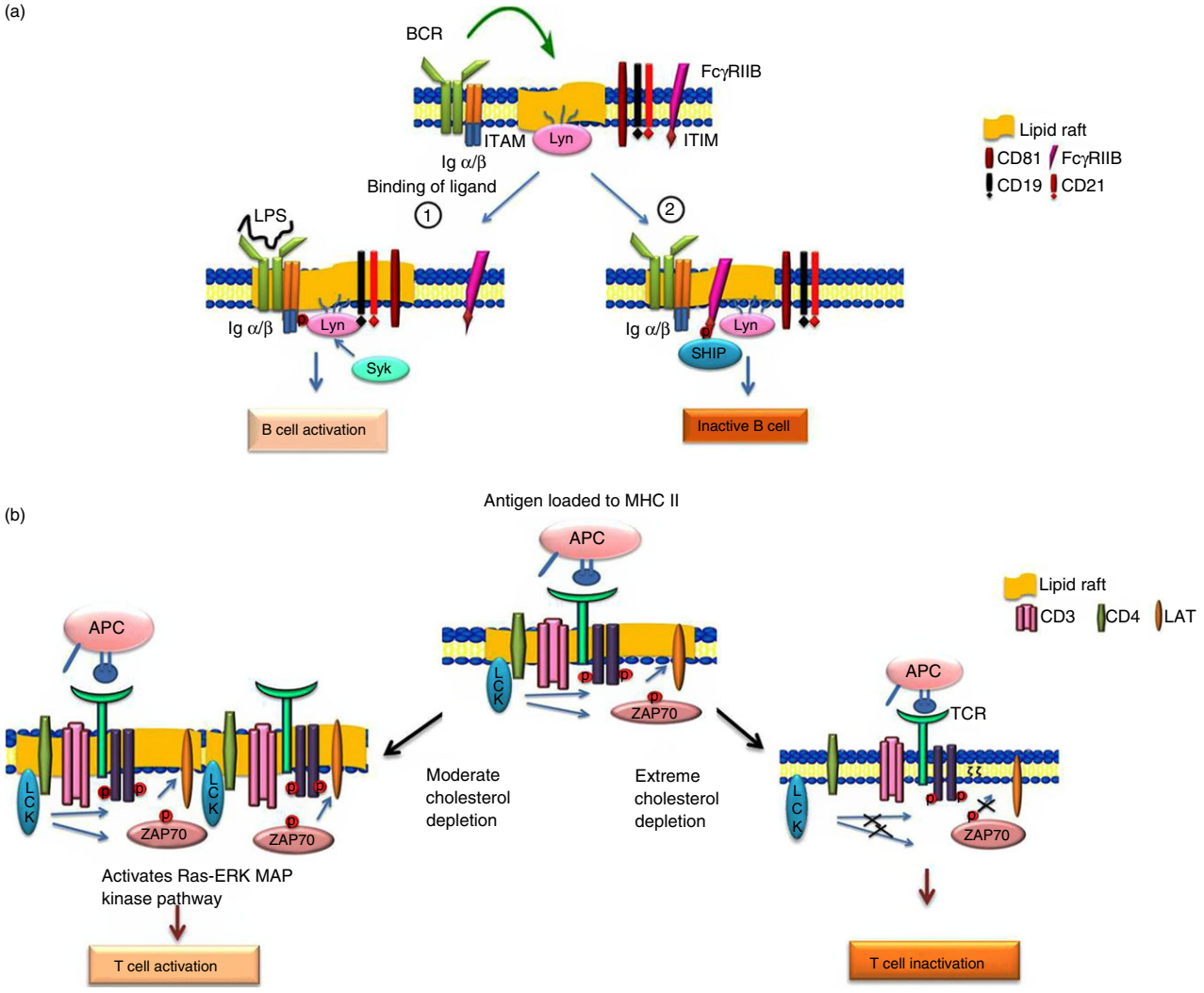

Figure 4. (a) The role of lipid rafts in B-cell activation. Ligand binding localizes the B-cell receptor (BCR) to lipid rafts where Lyn phosphorylates the immunoreceptor tyrosine-based activation (ITAM) domain of BCR and recruits Syk and other molecules which lead to B-cell activation. (1) In the presence of ligand (lipopolysaccharide; LPS), various co-receptors such as CD19, CD21 and CD81 associate with the raft domain and positively regulate B-cell signalling. (2) Interaction of the BCR with FcγRIIB co-receptor leads to phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) domain, which recruits SH2 domain containing inositol 5-phosphatase (SHIP) phosphatase in the raft domain and negatively regulates B-cell activation. (b) The role of lipid rafts in T-cell activation. Cross-linking of antigen loaded onto the MHC II molecule on antigen-presenting cells (APC) with the T-cell receptor (TCR) leads to TCR–CD3 translocation to lipid rafts where lymphocyte-specific protein tyrosine kinase (Lck) not only phosphorylates the ITAM domain present on ζ chain of TCR but also phosphorylates ZAP70, both of which bind to the phosphorylated domain and activate LAT, resulting in T-cell activation. Moderate cholesterol depletion leads to raft-clustering and activation of the Ras–extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase resulting in T-cell signalling while extreme cholesterol depletion alters T-cell activation by affecting the localization and phosphorylation of Lck, LAT and ZAP 70.

non-lipid raft domains in the resting state and translocate to lipid rafts during B-cell activation. These co-receptors decrease the internalization of BCR and lead to prolonged persistence of BCR in lipid rafts and sustained signalling as detected by co-localization of BCR with the DiIC16 raft marker.[68] On the other hand, the low-affinity co-receptor FcγRIIB inhibits B-cell activation when cross-linked with BCR in rafts.[69] During BCR cross-linking, the immunoreceptor tyrosine-based inhibitory motif in the cytoplasmic domain of FcγRIIB becomes phosphorylated and recruits SH2 domain-containing inositol phosphatase, which further dephosphorylates the ITAM domain and so inhibits B-cell activation.[70]

Biochemical and microscopy studies have revealed the localization of the T-cell receptor (TCR) outside the raft domain in a resting state and its translocation in the detergent-resistant membrane fraction during T-cell activation.[71] In contrast, Dinic *et al.*[72] found TCR in the

raft domains of resting T cells and the aggregation of these domains upon TCR engagement. The disparate results concerning the association of TCR with lipid rafts is probably due to the differing methodologies used for the study. T-cell signalling involves the molecules Lck (lymphocyte-specific protein tyrosine kinase), dually acylated Src-family kinase and LAT, which are constitutively present in the rafts. The TCR comprises an α/β heterodimer that associates with CD3 and the ζ homodimer. The α/β chain comprises the ligand-binding site while CD3 and the ζ chain have a cytoplasmic ITAM domain that becomes phosphorylated by Lck. Upon T-cell stimulation, the tyrosine kinase ZAP70 binds to the phosphorylated ITAM in rafts and activates LAT and recruits other signalling scaffolds for T-cell activation. Moreover, the regulators of Lck – protein tyrosine kinase Csk (C-terminal Src kinase) and the protein tyrosine phosphatase CD45 – are also compartmentalized in T-cell signalling. Csk interacts with Cbp (Csk-binding protein) a raft protein for regulating Lck activity whereas CD45 is excluded from the lipid rafts during T-cell activation.[73]

There are several pieces of evidence showing that lipid rafts are not only required but are also important for T-cell activation. A mutant form of Lck that is excluded from the raft domain is unable to activate TCR signalling.[74] Lipid raft disruption by methyl-β-cyclodextrin modulates T-cell activation by affecting the phosphorylation and localization of Lck, ZAP-70 and LAT as shown in Fig. 4(b).[75] On the other hand, a few studies have reported that moderate cholesterol depletion activates T-cell signalling by lipid raft aggregation and activation of the Ras–extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase pathway.[76,77] This discrepancy could be due to the variation in the time duration of MBCD treatment and the extent of cholesterol depletion, which affects cell viability.

The role of lipid rafts in segregation and integration of key signalling molecules (cytoplasmic CD45, phospholipase Cγ1, SHP1) in T-cell activation have been proven by artificially targeting them individually.[78] Another raft protein, raftlin was also found to play an important role in B-cell as well as T-cell signalling.[79] Moreover, lipid rafts were found to be crucial for T-cell polarization as reduced polarization to T helper type 17 cells was observed while differentiation to the other T-cell subtypes T helper type 1, T helper type 2 and regulatory T cells was not affected when the level of lipid raft glycosphingolipid was decreased in $CD4^+$ T cells by using specific inhibitors of glucosylceramide synthase.[80]

B-cell–T-cell interactions lead to polarization and clustering of TCR and signalling molecules along with the lipid rafts at the immunological synapse, which is required for enhanced and sustained downstream signalling in both B and T cells. CD4 partitions into lipid rafts because of its lipid modification and this induces raft aggregation and formation of molecular clusters at the site of the immunological synapse whereas $CD8^+$ T cells did not show this type of lipid raft polarization upon stimulation.[81] Co-receptors are known to enhance or impede the signalling by altering TCR association with raft domains. The CD28 co-receptor positively regulates T-cell activation by increasing cell surface lipid raft concentration as well as enhancing its localization to the immunological synapse, whereas CTLA4 (a negative regulator) inhibits intracellular transport to the T-cell surface.[82] Other co-stimulatory molecules CD2, CD5, CD9, CD26 and CD44 have also been found to enhance TCR association with lipid rafts.[83]

Lipid rafts alter cytokine signalling

Cytokines play a wide variety of roles from proliferation, differentiation, migration and apoptosis of immune and non-immune cells to self-renewal of embryonic stem cells. Cytokine signalling is regulated by compartmentalization of multi-subunit chains of cytokine receptors in the raft domains, which determines the specificity of the signalling and provides an efficient platform for concentrating kinases and adaptor molecules. Disruption of lipid rafts on cells alters cytokine signalling and so attenuates the cytokine response.

The main receptor that transduces TNF-α signals inside the cell is TNF receptor 1 (TNFR1) and there are contradicting reports on its localization in the lipid raft domain. In the absence of stimulation, a small fraction of endogenous TNFR1 is localized to lipid rafts while the majority of the fraction is present in the non-raft domain. Binding of a ligand recruited more fractions of TNFR1 to lipid rafts in the HT1080 fibrosarcoma cell line and HeLa cells.[84] However, TNFR1 was not detected in lipid rafts of resting fibroblasts whereas in U937 and NIH3T3 cells TNFR1 localized exclusively to lipid rafts even in unstimulated conditions.[85] These anomalies could be due to the differences in lipid raft isolation methods. Upon ligand binding, the TNF receptor-associated death domain adaptor binds to the intracellular domain of the receptor and recruits TRAF2 and receptor-interacting protein kinase to the raft domain, which further leads to downstream activation of NF-κB and to cell survival.

Treatment of HT1080 cells with MBCD inhibited TNF-α-induced NF-κB activation and rendered these cells susceptible to apoptosis. Doan *et al.*[86] reported that TNFR1 is capable of initiating signalling in both raft and non-raft membrane fractions. Raft TNFR1 initiates p42mapk/ERK2 signalling while non-raft TNFR1 initiates NF-κB signalling. Hence, TNF-α-induced p42mapk/ERK2 activation was dependent on lipid raft integrity while the activation of NF-κB occurs independently of cholesterol

and lipid raft integrity. Moreover, TNFR1 contains a specific amino acid sequence in the death domain which is required and sufficient for their translocation to lipid rafts. Deletion of these sequences leads to loss of raft localization and uniform distribution of the receptor in the plasma membrane.[87]

Fas, a member of the TNFR superfamily was found to be constitutively present in lipid rafts of thymocytes and the B-cell line SKW6.4 whereas other studies reported that FasL-induced apoptosis was independent of lipid rafts in Jurkat and HT1080 cells.[88] Another member of the TNFR superfamily, CD40, is involved in mediating immune and inflammatory responses like the development of memory B cells and antibody class switching. CD40 interacts with TRAF2 and TRAF3 in rafts and activates NF-κB. Hostager[89] observed that cholesterol depletion inhibits translocation of TRAF molecules to rafts and so alters CD40 signalling.

Interferons are key players in innate and acquired immune responses against viral infections and have antiproliferative ability. Decreased interferon-γ (IFN-γ) signalling was observed in *Leishmania donovani*-infected macrophages of Kala-azar patients. Sen *et al.*[90] showed that *L. donovani* increases membrane fluidity and perturbs IFNGR1 and IFNGR2 subunit assembly of the receptor, which occurs in lipid rafts in normal macrophages. Restoration of macrophage membrane cholesterol by exogenous liposomal delivery restores IFN-γ signalling. Binding of IFN-γ to the receptor activates receptor-associated Janus kinase, which in turn phosphorylates Stat (signal transducer and activator of transcription) in the caveolar membrane.[91]

Leukaemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) are IL-6 family member cytokines known to have neuroprotective functions. The literature reveals that CNTFR is present in the detergent-resistant membrane fractions as it is attached by a glycosylphosphatidylinositol anchor to the extracellular membrane while LIFR and gp130 are transmembrane proteins not residing in raft domain.[92] LIF interacts with the low-affinity receptor (LIFR) and gp130 to transduce their signals while CNTF requires CNTFR along with LIFR and gp130 for signal transduction. Stimulation of IMR-32 neuronal cells with CNTF resulted in translocation of LIFR and gp130 to lipid rafts and cholesterol depletion inhibited this translocation.[93] Lee *et al.*[94] observed that depletion of cholesterol by MBCD in embryonic stem cells resulted in decreased expression of LIF-induced pluripotency genes *Oct4*, *Sox2*, *Rex1* and *FoxD3* and so showed the involvement of lipid rafts in LIF-induced embryonic stem (ES) cell renewal.

Transforming growth factor (TGF-β) regulates proliferation and differentiation of the cell. Receptor complex for TGF-β signalling (TβR1) and (TβR2) have both been reported to be present in lipid rafts in epithelial cells.[95] On ligand binding, TβR2 phosphorylates TβR1, which in turn activates Smad proteins, MAP kinase and phosphoinositide 3 kinase molecules in rafts and leads to epithelial to mesenchymal transition. Zuo *et al.*[96] found that nystatin, a cholesterol-sequestering agent, inhibits TGF-β-induced epithelial to mesenchymal transition in epithelial cells by inhibiting the localization of TGF-β receptors in lipid rafts and activation of the MAP kinase pathway.

Role of lipid rafts in autoimmune disorders

Systemic lupus erythematosus (SLE) and rheumatoid arthritis are autoimmune disorders characterized by impaired humoral and cellular immune responses to self-antigens. The underlying molecular mechanism involves impaired TCR signalling in SLE patients. Peripheral blood T cells isolated from SLE patients were found to have more cholesterol and GM1 content in their plasma membrane compared with healthy individuals. This suggests an activated state of T cells in SLE, as T cells activated *in vitro* synthesize more GM1. Reports also suggest that aggregated lipid rafts present on the T-cell surface contain TCR as well as other co-stimulatory molecules. Differential expression and localization of CD45 tyrosine phosphatase, CD3 and Lck in lipid rafts were increased in T cells from SLE patients.[97] Hence, lipid rafts could be considered as a therapeutic target in SLE treatment. Deng *et al.*[98] reported that disruption of lipid rafts by cyclodextrin delayed disease progression whereas aggregation of rafts using cholera toxin accelerates disease progression in mice.

Rheumatoid arthritis is a chronic, systemic inflammatory disease that mainly affects synovial joints and leads to a disabling and painful condition. The synovium of the inflamed joints harbours many responsive T cells along with activated neutrophils, macrophages, B lymphocytes and dendritic cells. Grinnell *et al.*[99] observed that T cells isolated from synovial fluid of inflamed joints of rheumatoid arthritis patients had an activated phenotype but were hyporesponsive to stimuli compared with T cells from healthy individuals. This hyporesponsiveness of T cells is due to dysfunctional TCR signalling initiated by an increase in oxidative stress in inflamed joints due to the generation of free radicals and depletion of antioxidant glutathione. Under oxidative stress, LAT becomes dissociated from the raft domain, which ultimately leads to abrogation of TCR signalling.[100] This signifies the importance of localization of T-cell signalling components in lipid rafts for efficient T-cell activation.

Conclusion and future perspective

The integrity of rafts and spatial segregation of receptors in rafts regulates a variety of signal transduction pathways. Specific targeting of lipid rafts could be used as a

potential therapeutic target in inflammatory and autoimmune diseases. This review summarizes the existing evidence on the involvement of lipid rafts in immune signalling.

Rational targeting of membrane rafts or caveolae and perturbing their interactions with signalling molecules may soon become a promising therapy for treatment and cure of diseases. To our knowledge, no previous report has addressed the possible association of microRNA with lipid rafts in human diseases. Furthermore, it may be important to develop techniques suitable for studies of membrane microdomains in other physiological functions. Further studies are required to understand the role of lipid rafts in the pathogenesis of other immune disorders.

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