LIPID RAFTS AND B-CELL ACTIVATION

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The B-cell antigen receptor acts during B-cell activation both to initiate signalling cascades and to transport antigen into the cell for subsequent processing and presentation. Recent evidence indicates that membrane microdomains, termed lipid rafts, have a role in B-cell activation as platforms for B-cell receptor (BCR) signalling and might also act in antigen trafficking. Lipid rafts might facilitate the regulation of the BCR during B-cell development by B-cell co-receptors, and during viral infection. So, lipid rafts seem to be an important new piece of the B-cell signalling puzzle.

ITAM
Immunoreceptor tyrosine-based activation motif, which is present in the cytoplasmic domains of the BCR Igα–Igβ complex and becomes tyrosine phosphorylated by Lyn after BCR activation.

The B-cell receptor (BCR) has two interrelated functions in B-cell activation. The first is to initiate signal cascades that result in the transcription of a variety of genes associated with B-cell activation[1,2] (FIG. 1). However, signalling through the BCR alone is insufficient for full activation, and the B-cell response to most antigens requires cognate interactions with antigen-specific T-helper cells[3]. The second is the uptake and targeting of antigen to the major histocompatibility complex (MHC) class II antigen processing and presentation pathway. The signalling and antigen-transport functions of the BCR are interdependent, in that BCR signalling is necessary for the correct and rapid targeting of the antigen to the MHC-class-II-containing compartments[4]. Indeed, in the case of signalling-defective BCRs, either antigen is not processed or the rate of antigen delivery is significantly reduced. In turn, the internalization of the BCR might have an important role in regulating signalling by removing activated receptors from the cell surface. Given the intimate relationship between BCR signalling and antigen transport, these activities are thought to be carefully coordinated in B cells by mechanisms that remain to be discovered.

The signalling cascades initiated by antigen binding to the BCR are now understood in significant biochemical detail[1,2]. The BCR contains an antigen-binding immunoglobulin molecule that has no direct signalling function. Signalling is mediated by an associated heterodimer, Igα–Igβ, which contains in its cytoplasmic domain a immunoreceptor tyrosine-based activation motif (ITAM). Recent evidence indicates that the BCR is composed of one immunoglobulin and one Igα–Igβ complex and might reside on the B-cell membrane as a dimer or a multimer[5]. On BCR crosslinking by multivalent antigens, the ITAMs become phosphorylated by the Src-family kinase Lyn, providing a binding site for the SH2-domain-containing kinase Syk, triggering the signalling cascades. Although the cascade that follows phosphorylation of the BCR by Lyn has been resolved in considerable detail, the event that initiates the association of the antigen-crosslinked BCR with Lyn is not known.

Far less is known about the mechanism by which the BCR is internalized after antigen crosslinking, either to target antigen for processing or to downregulate signalling. Indeed, it is not known whether BCR internalization is via the well-characterized clathrin- or ubiquitin-mediated pathways, nor even whether there are one or more pathways for internalization. A better understanding of the molecular mechanisms underlying BCR internalization might indicate mechanisms by which signalling influences internalization and vice versa.

Lipid rafts in BCR function

A clue to how the antigen-crosslinked BCR becomes associated with Lyn came from studies in cell biology that indicated that the cell surface contained membrane microdomains, termed lipid rafts, that act as platforms for receptor signalling and trafficking[6] (BOX 1).

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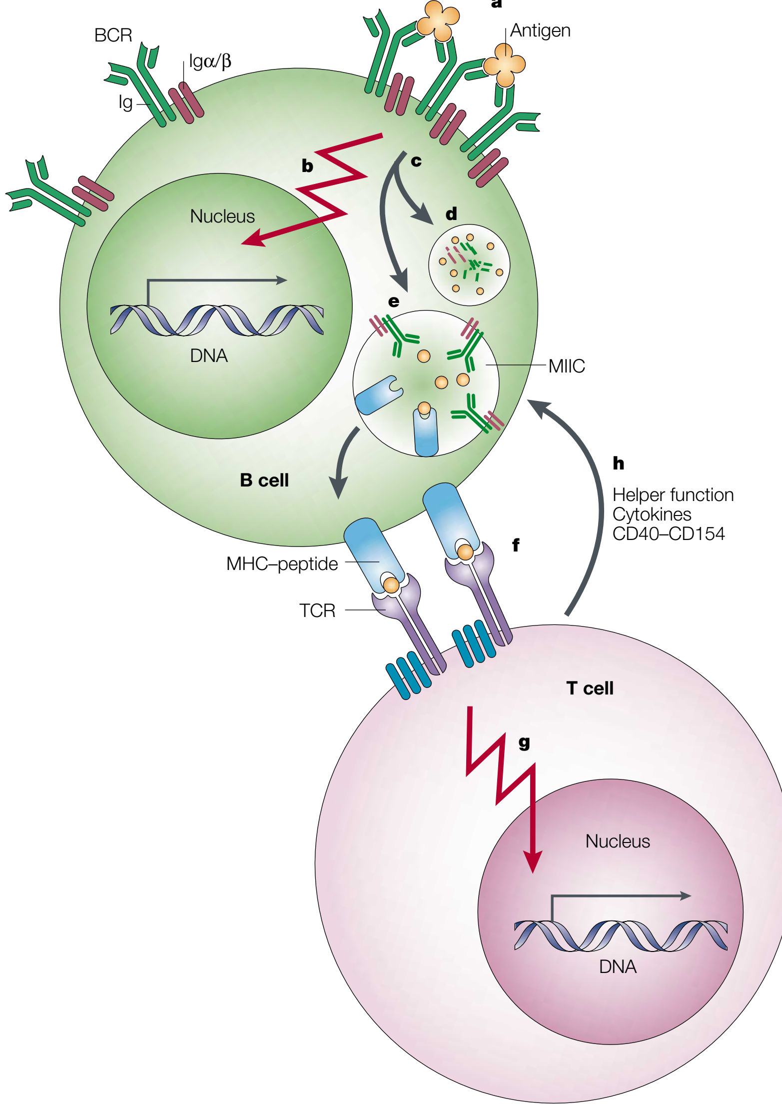

Figure 1 | **The function of the BCR in B-cell activation.** Following antigen binding (**a**), the B-cell receptor (BCR) triggers a signal-transduction cascade (**b**), which leads to the transcriptional activation of genes associated with B-cell activation. The BCR is internalized (**c**) and either degraded (**d**) or trafficked to an intracellular compartment termed the MIIC (**e**), where newly synthesized major histocompatibility complex class II (MHC) molecules and peptides derived from the antigen bound to the BCR are formed into complexes. The antigen-processing and BCR-degradation pathway might not be identical and are shown here to occur in two different endosomal compartments. The peptide–MHC complexes are subsequently transported to the cell surface, where they are recognized by the T-cell receptor (TCR) of T-helper cells (**f**), leading to T-cell activation (**g**). The activated T cell provides 'help' to the B cell, leading to full B-cell activation (**h**) through both secreted cytokines and cell–cell interactions mediated by receptor pairs such as CD40–CD154. Ig, immunoglobulin.

Significantly, rafts were shown to concentrate the doubly acetylated Src-family kinases[7]. The description of rafts immediately indicated a mechanism to regulate the association of the BCR and Lyn. It was proposed that the BCR in resting cells is excluded from rafts that concentrate Lyn but that, on antigen binding and oligomerization, the BCR translocates into rafts, where it is phosphorylated by Lyn, initiating a signal cascade and antigen targeting[8]. Indeed, using detergent solubilization to isolate rafts, the BCRs in resting B cells were shown to be excluded from lipid rafts that concentrate Lyn[8–11]. Rafts also concentrate the signalling components c-Abl[12], PAG/cbp (a membrane protein involved in the regulation of Src family kinases[13,14]), actin[8,11] and ezrin[11]. Most membrane proteins are excluded from rafts, including the tyrosine phosphatase CD45 (REF. 8) and CD22 (REF. 10), a cell-surface receptor that regulates B-cell signalling by recruiting the potent phosphotyrosine phosphatase SHP1.

On crosslinking by antigen, the BCR immediately becomes associated with lipid rafts, even at 4 °C (REFS 8–11). However, this translocation is transient: 15–30 min after activation, the BCR is no longer detected in rafts. The translocation of the BCR into rafts depends on the presence of cholesterol in the membrane and presumably on the integrity of the lipid rafts, which disperse after cholesterol depletion[8,9,11]. Using radiolabelled antigen, the proportion of BCR that associates with the raft was estimated to be, at most, 30–40% (REF. 8). However, because this estimate was based on detergent solubilization, it is not possible to know whether this 30–40% represents the actual proportion of the BCR that became raft associated or whether it reflects the efficiency with which the rafts were isolated.

The BCR present in lipid rafts after crosslinking is phosphorylated, as is Lyn[8–10]. Crosslinking of the BCR also results in the recruitment to rafts of several proteins that are involved in the BCR signalling cascade, including Syk, Btk, Vav, SHIP, phospholipase Cγ2, phosphatidylinositol 3-kinase and BLNK[8,9,11,15], indicating that rafts are sites of BCR signalling. Although antigen binding is required to induce a significant number of BCRs to associate with rafts, an observation of potential importance is that a small proportion of the BCR seems to be constitutively present in rafts[10,16], indicating that the unligated BCR might have a weak affinity for rafts. The function of the BCR constitutively present in rafts is not known but could involve signalling for cell survival.

At present, less is known about the relationship between rafts and BCR internalization. A label attached to a ganglioside component of rafts (GM1) was found in the MHC-class-II–peptide-loading compartment (MIIC) after BCR crosslinking but not in resting B cells[8], providing evidence that the BCR that is associated with raft components is trafficked to the MIIC. However, a protein associated with the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage, and so constitutively present in rafts, does not internalize to the MIIC after crosslinking of the BCR. This indicates that either the entire raft is not internalized or a sorting event precedes BCR internalization.

So, based on the work of several investigators, a model has been proposed (FIG. 2) in which the BCR in resting cells has little affinity for rafts and so is, for the most part, excluded from rafts. Crosslinking the BCR results in a change in the BCR that increases its affinity for the rafts, resulting in the stable residency of the receptors in rafts. In the rafts, the BCR is brought into association with Lyn, which phosphorylates Igα–Igβ and initiates the signalling cascade, resulting in the recruitment of additional signalling components.

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The BCR is a member of the multichain immune recognition receptor family that includes the T-cell receptor for antigen (TCR) and the high-affinity receptor for IgE (FcεR1). The members of this receptor family share a common mechanism for the initiation of signalling involving Src-family kinases. The evidence so far indicates that rafts have a role in initiating signalling for both the TCR and the FcεR1 (REF. 17). Rafts might therefore have important roles in the spatial organization of this family of receptors during immune-cell activation.

After triggering of the signalling cascade, the BCR (associated with GM1) is internalized by the cell. At present, it is difficult to determine whether the BCR is internalized directly from rafts or whether the BCR first moves laterally in the membrane, associated with GM1, and is then internalized by a raft-independent mechanism. This will be an important point to resolve to understand the regulation of BCR trafficking.

At present, the proposed model is based only on data from experiments in which raft components were identified by their detergent insolubility. Obvious shortcomings of this approach include the possibilities that the detergent-insoluble fractions do not reflect the nature of the membranes in living cells and that artefacts might be caused if the detergent concentrations or the temperatures of the isolation conditions differ between studies. However, recent studies in other cell types using a variety of advanced technologies (including FLUORESCENCE RESONANCE ENERGY TRANSFER[18], SINGLE FLUOROPHORE TRACKING MICROSCOPY[19] and PHOTONIC FORCE MICROSCOPY[20]) have provided convincing evidence for the existence of rafts in living cells. Although the judicial use of detergent solubility can be a powerful tool for characterizing rafts, techniques to measure protein–protein and protein–lipid interactions in living cells will be necessary to determine details of the relationship between the BCR and rafts.

FLUORESCENCE RESONANCE ENERGY TRANSFER
A method to determine whether proteins are close to one another by measuring the loss of fluorescence anisotropy between fluorophores that are associated with the proteins.

SINGLE FLUOROPHORE TRACKING MICROSCOPY
A method to measure the lateral motion of a single fluorescently labelled molecule in the plasma membrane using single dye tracking. It yields information about the diffusion and dynamics of individual raft proteins and lipids.

PHOTONIC FORCE MICROSCOPY
A method to measure the local viscous drag of a single membrane protein using a laser trap. It yields information about the size and dynamics of individual rafts.

Box 1 | What are lipid rafts?

Lipid rafts are sphingolipid- and cholesterol-rich membrane microdomains in the outer leaflet of the plasma membrane. The plasma membrane is composed primarily of sphingolipids, (glycerol)phospholipids and cholesterol. Sphingolipids differ from most phospholipids in that they have long, largely saturated acyl chains that allow them to pack tightly in a bilayer, forming a gel phase in which there is very little lateral movement or diffusion. The gel phase of the sphingolipids is altered by the association of cholesterol, which condenses the packing of the sphingolipids by occupying the spaces between the acyl chains. So, cholesterol-containing sphingolipid microdomains exist in a liquid-ordered phase that is significantly more fluid than the gel phase.

By contrast, phospholipids are rich in unsaturated acyl chains that tend to be kinked and consequently to pack loosely into a liquid-disordered phase that is considerably more fluid, allowing rapid lateral movement within the bilayer. The different packing of the sphingolipids and phospholipids probably leads to their phase separation in membrane bilayers. Sphingolipid microdomains float in a phospholipid bilayer, leading to the coining of the term 'lipid rafts'. Cholesterol preferentially partitions into the liquid-ordered phase rather than the liquid-disordered phospholipid bilayer and is essential for the maintenance of the two phases. Extracting cholesterol from the membrane results in the dispersion of lipid rafts.

The membrane outer leaflet rafts are believed to be linked to an inner leaflet that is probably rich in phospholipids with saturated fatty acids and cholesterol. The size of rafts and their lifetimes in the membranes of resting cells are uncertain. Current evidence indicates that the elemental rafts might be small (26–70 nm in diameter)[21,47], containing only several thousand molecules and therefore accommodating only a few proteins. Estimates of the half-life of rafts range from milliseconds to minutes. The initial characterization of the components of rafts took advantage of the observation that rafts were insoluble in certain nonionic detergents and so could be separated from the glycerolipid-containing membranes. Rafts were shown selectively to include some proteins and to exclude others, so rafts provide a mechanism for the lateral sorting of proteins in the membrane.

R, R', Hydrocarbon chains of fatty acids
R'', Head group
GPI, glycosylphosphatidyl inositol

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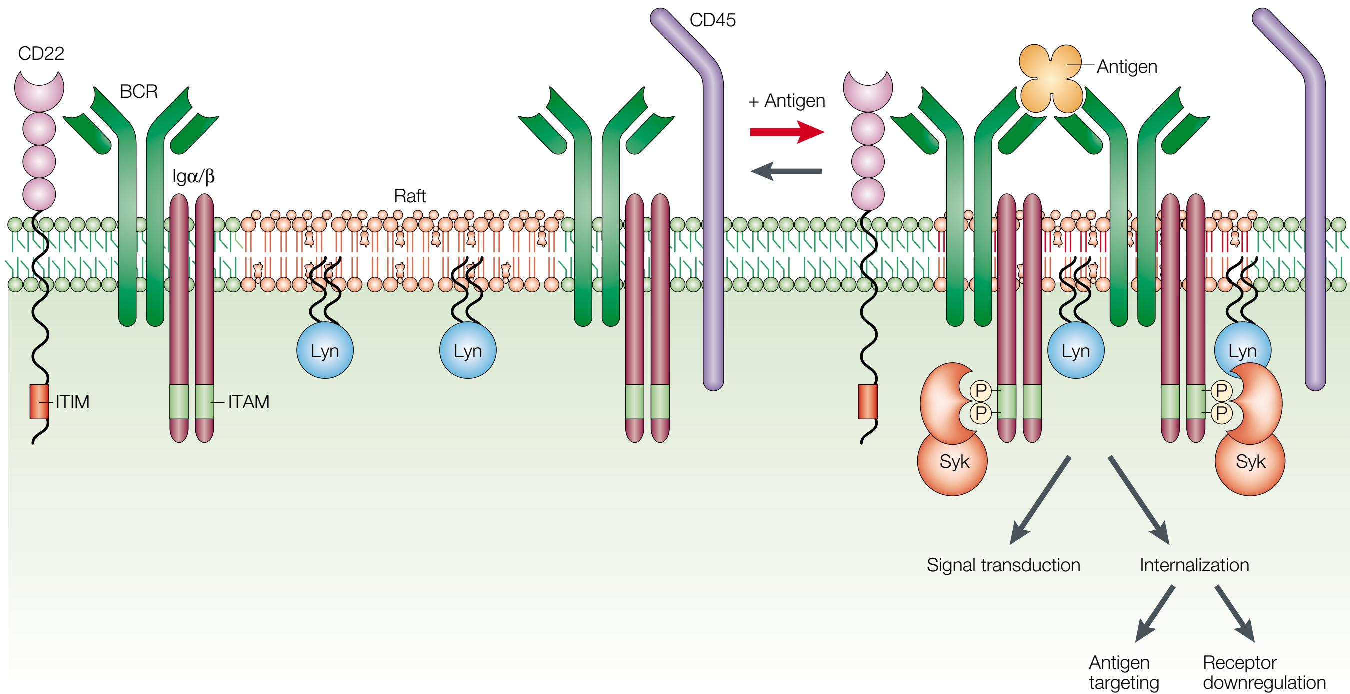

Figure 2 | **Model of the role for lipid rafts in B-cell activation.** In resting B cells, the B-cell receptor (BCR) is excluded from rafts, which concentrate the Src-family kinase Lyn. Most other membrane proteins are also excluded from rafts, including the negative regulators of B-cell function CD22 and CD45. In the absence of antigen, the BCR monomer has a weak affinity for the rafts, but multivalent antigen binding oligomerizes the BCR, increasing affinity for the rafts. Stable residency in the rafts results in association with Lyn, which phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs) of the BCR, recruiting Syk and initiating signalling cascades. The BCR might either be internalized directly from rafts or move laterally from rafts and then be internalized for the purpose of antigen processing or downregulation. ITIM, immunoreceptor tyrosine-based inhibitory motif.

Molecular mechanism of BCR–raft association

A key feature of rafts in B cells is their selective inclusion and exclusion of various membrane components. The rules governing the constitutive association of proteins with rafts are just beginning to be revealed[6]. In general, certain classes of proteins are concentrated in rafts, including GPI-linked proteins, doubly acetylated and palmitoylated proteins, and the α subunits of G-protein-coupled receptors, whereas most membrane proteins are excluded. So, in B cells, Lyn is associated with rafts, owing to its myristoylation and palmitoylation. Similarly, PAG/cbp is a palmitoylated transmembrane protein that is associated with rafts. At present, it is not known whether rafts are homogeneous in composition. Current evidence indicates that the elemental rafts in resting cells are small, ranging in size from <100 molecules to several thousand molecules[21]; if this is so, an individual raft might not contain all the molecules that are constitutively associated with rafts in the cell membrane. Consequently, only a subset of the cell's heterogeneous rafts might function as signalling or trafficking platforms for any given receptor.

A key feature of the model depicted in FIG. 2 is its prediction that antigen binding induces a change in the BCR that results in its association with rafts. Studies are just beginning to address the nature of that change and there remains a great deal to be learned. The association of the BCR with rafts requires antigen binding but, at present, there is little information on the effects of the affinity, valency or conformation of the antigen on its ability to induce raft association. The ability of the BCR to associate with rafts after crosslinking is not a characteristic of all B-cell surface receptors, but the structural characteristic of the BCR that allows its inducible association with rafts is not known. Studies of the FcεR1, which also becomes raft associated after crosslinking, indicate that the transmembrane region of the receptor is crucial[22]. Consistent with this is the observation that BCRs that contain mutations in the transmembrane region still translocate into rafts upon crosslinking, but maximal translocation requires more time and higher temperatures[16]. The observation that the acetylation or palmitoylation of proteins promotes raft association indicates a mechanism for the induced association of the BCR with rafts. However, at present, there is little evidence to suggest that the BCR is modified with lipids after activation.

At least two of the earliest events after antigen binding (the phosphorylation of the BCR and its association with the actin cytoskeleton) do not seem to be required for raft association. This means that BCRs that are defective in signalling or blocked by Src-family kinase inhibitors can be induced to translocate into rafts[10,16]. Similarly, treating cells with inhibitors of the actin cytoskeleton does not block antigen-induced BCR translocation into lipid rafts[16]. Significantly, although the BCR translocates into rafts in the absence of receptor phosphorylation or an intact actin cytoskeleton, the

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ANERGY
A state of non-reactivity in B cells that is induced by antigen exposure, commonly in immature B cells

receptor seems to be less stably associated with the rafts under such conditions[16]. This indicates that active signalling might be needed to stabilize the BCR in rafts. Indeed, it has been suggested for other cell types that, after the initial association of the receptor with rafts, the rafts cluster to form larger domains[6]. For the BCR, clustering might be a crucial step in establishing a stable signalling domain. Raft clustering could be mediated by a multivalent antigen, by association of BCR in rafts with the actin cytoskeleton or by adaptor proteins recruited to the BCR after its initial phosphorylation.

Taken together, the results so far indicate that the initiating event in B-cell activation might simply be the antigen-driven oligomerization of the BCR to achieve a conformation with increased affinity for rafts (FIG. 2). This increased affinity would shift the equilibrium of the BCR towards the rafts, where Lyn-dependent signalling cascades are initiated. In this model, the initial step in B-cell activation is strictly an antigen-sensing event, which would depend on the valency of the antigen and its affinity for the BCR. Any factor that affected these parameters would be anticipated to influence B-cell activation.

Regulating BCR–raft association

If the association of the BCR with rafts is a crucial initial step in B-cell activation, factors that influence signalling or dictate the outcome of antigen engagement by B cells might regulate the association of the BCR with rafts. If so, an analysis of the mechanisms by which the association of the BCR with rafts can be influenced has the potential to reveal a great deal about the molecular mechanisms that underlie the association itself. As described below, BCR–raft association is influenced during development, by co-receptors and by viral infection (BOX 2).

Function of rafts during B-cell development. To a large extent, the developmental state of the B cell dictates the outcome of the engagement of the BCR with antigen[1,23,24] (FIG. 3). In pre-B cells, the immunoglobulin heavy-chain locus undergoes V–D–J rearrangements and, if the rearrangements are successful, this results in the expression on the cell surface of a pre-BCR that is composed of the immunoglobulin heavy chain bound to a surrogate light-chain complex. The cell-surface expression of the pre-BCR is necessary to signal for further development. The mechanism by which signalling is initiated is not known, nor is it known whether pre-BCR signalling requires a ligand. If the pre-BCR is expressed, development proceeds to the immature-B-cell stage, during which a BCR containing a μ heavy chain and a rearranged light chain is expressed on the surface. Antigen encounter at the immature stage eliminates self-reactive B cells by apoptosis or ANERGY. If the B cell does not encounter antigen at the immature stage, it progresses to the mature-B-cell stage, during which encounter with antigen leads to activation.

The evidence indicates that the association of the BCR with lipid rafts changes during development (FIG. 3). As described above, in mature resting B cells, the BCR is excluded from lipid rafts and antigen binding results in the association of the BCR with rafts. By contrast, in immature B cells, antigen binding to the BCR does not induce stable association of the BCR with rafts and the BCR signals for apoptosis outside rafts[25,26]. A similar phenomenon has been described for the behaviour of the BCR in B cells that have been rendered unresponsive or anergic by chronic exposure to antigen[10]. So, the failure of the BCR to associate with lipid rafts correlates with the failure of the BCR to signal for activation. In pre-B cells, a significant proportion of the BCR is constitutively associated with rafts[15]. This is in contrast to mature B cells, in which only a small amount of BCR is raft associated in the absence of antigen. Although the signalling function of the pre-BCR is not known, it is interesting to speculate that the raft-associated pre-BCR might generate a survival signal that is required to drive development.

The theme emerging from these studies is therefore one in which the access of the BCR to rafts is altered during development in order to alter the functional outcome of signalling. The mechanisms by which access of the BCR to rafts is limited is not known and might involve developmental changes in the rafts themselves that alter the affinity of the BCR for the rafts or, alternatively, might involve changes in the non-raft regions of the membrane that either restrict the mobility of the BCR or influence the ability of the BCR to form

Box 2 | Function of lipid rafts during EBV infection

If lipid rafts are central to B-cell activation, their function might be subverted by viruses to allow infection of the B cell or to block the antibody response to the virus. The best-studied example of the intimate relationship between lipid rafts and virus infection is Epstein–Barr virus (EBV) infection of B cells. EBV is a human oncogenic herpes virus that can readily transform primary B cells *in vitro* but, in most individuals, establishes a latent infection[48].

LMP2A is an EBV-encoded integral membrane protein that is associated with latency in B cells[49]. LMP2A has a large cytoplasmic domain that contains an immunoreceptor tyrosine-based activation motif (ITAM) and a binding site for Lyn, and has been shown to block B-cell receptor (BCR) signalling. BCR signalling has been shown to induce EBV replication in latently infected cells, and so the blockade of BCR signalling by LMP2A might be essential to maintain latency. LMP2A also seems constitutively to generate signals, as shown in a transgenic mouse model in which LMP2A could replace many of the signalling functions of the pre-BCR in early B-cell development[50]. The constitutive signalling emanating from LMP2A might be necessary for latency[49]. Recent results indicate that LMP2A is constitutively present in the rafts of latently infected B cells[51,52] and blocks the translocation of the BCR into rafts[51]. As a consequence, the BCR neither signals nor is internalized. The Lyn-binding site of the LMP2A cytoplasmic domain is required to block BCR entry into rafts[51], indicating that the LMP2A block is not due to simple steric hindrance nor to physical exclusion of the BCR from rafts. LMP2A and mutant LMP2A proteins should provide powerful tools to dissect the mechanism by which the BCR associates with rafts.

The EBV-encoded gene product LMP1 is an integral membrane protein that is associated with growth transformation in infected B cells[53] and seems to generate signals that mimic those of CD40, a tumour necrosis factor (TNF) family member[54]. After ligand binding, CD40 translocates into lipid rafts in B cells, where it recruits the TNF-receptor-associated factors (TRAFs) to initiate signalling[55,56]. Recent studies showed that LMP1 is constitutively present in lipid rafts, where it signals and associates with the actin cytoskeleton through TRAF3 (REFS 52,56).

Therefore, at least two gene products of EBV seem to co-opt the function of rafts in B cells, presumably for the purpose of generating signals that are required for virus latency and growth transformation, and to interfere with the B cells' own signalling receptors.

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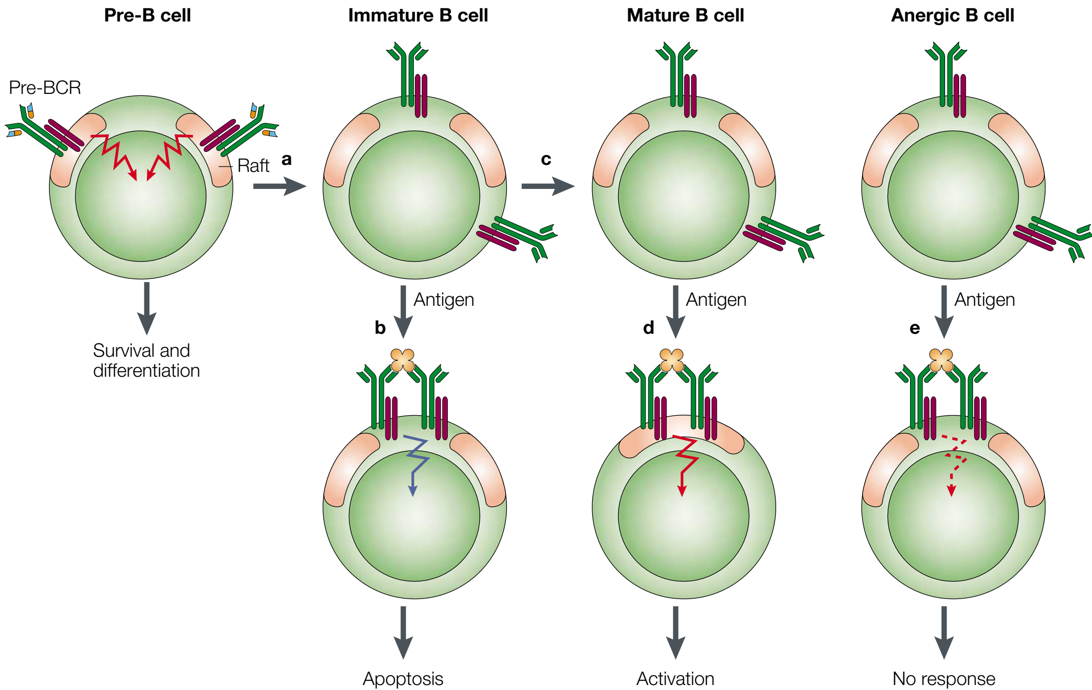

Figure 3 | **The B-cell receptor (BCR) and rafts in B-cell development.** In pre-B cells, the pre-BCR resides constitutively in lipid rafts and signals for light-chain rearrangement and continued development to the immature B-cell stage (**a**). In immature B cells, the BCR is excluded from rafts even after antigen binding that leads to apoptosis (**b**). If antigen is not encountered by the immature B cell, development continues to the mature B-cell stage (**c**). In mature B cells, antigen binding leads to association of the BCR with rafts and to B-cell activation (**d**). The BCR expressed by B cells that have been made tolerant or anergeric as a result of chronic exposure to antigen is excluded from lipid rafts even after antigen binding, which fails to activate the cells (**e**).

oligomers that have an increased affinity for rafts. Detailed comparisons of the components of rafts that are isolated from B cells at discrete developmental stages might provide insights into the mechanisms by which the association of the BCR with rafts is controlled.

Recently, evidence has been found that the association of the TCR with rafts also changes during development. The pre-TCR, composed of a rearranged β-chain associated with a surrogate α-chain, constitutively associates with rafts when pre-TCR signalling instructs the cell in lineage commitment[27]. In immature thymocytes, in which TCR engagement leads to apoptosis, the TCR fails to recruit lipid rafts[28].

Function of co-receptors and lipid rafts. The response of B cells to stimulation by antigen is modulated by a variety of co-receptors that have the capacity either to augment or to attenuate B-cell responses. Recent studies indicates that B-cell co-receptors act, at least in part, in lipid rafts and might influence the residency of the BCR in rafts. The co-receptor complex CD19–CD21 has been shown to augment signalling through the BCR[29]. CD19 contains a large cytoplasmic domain that is a specialized adaptor for the amplification of Src-family kinases and interacts with components of several different signalling pathways. CD19 is brought close to the BCR through the binding of complement-tagged antigens by CD21, a receptor for the C3d cleavage product of complement. So, C3d-tagged antigens bridge the BCR and the CD19–CD21 complex. Complement-tagged antigens are potent immunogens *in vivo*, inducing a maximal antibody response at concentrations 100–1,000 times lower than are required for unmodified antigens[30].

Recent studies have shown that the CD19–CD21 complex is excluded from rafts in resting B cells but that, when it is ligated to the BCR, it translocates into lipid rafts along with the BCR[31]. There are two repercussions of ligation of the BCR and the CD19–CD21 complex: a larger proportion of the BCR is stabilized in rafts, and the BCR persists in rafts for prolonged periods of time. The prolonged persistence in rafts correlates with prolonged signalling, as gauged by the phosphorylation of raft-associated proteins, including Igα and CD19. The mechanisms by which stable raft association is achieved are not known at present. The engagement of the CD19–CD21 complex and the BCR significantly decreases the internalization of the complex from the cell surface[31], indicating that CD19–CD21 might interfere with downregulation of the BCR. The wealth of mutant CD19 and CD21 proteins and knockout mice that been generated to analyse the function of this important regulatory complex should provide valuable tools for the dissection of the requirements for lipid-raft association.

At the opposite end of the spectrum in terms of BCR regulation is the low-affinity Fc receptor FcγRIIB. When this is ligated to the BCR through the binding of antigen–antibody complexes, it blocks BCR signalling[32]. Ligation of the BCR and the FcγRIIB also blocks BCR

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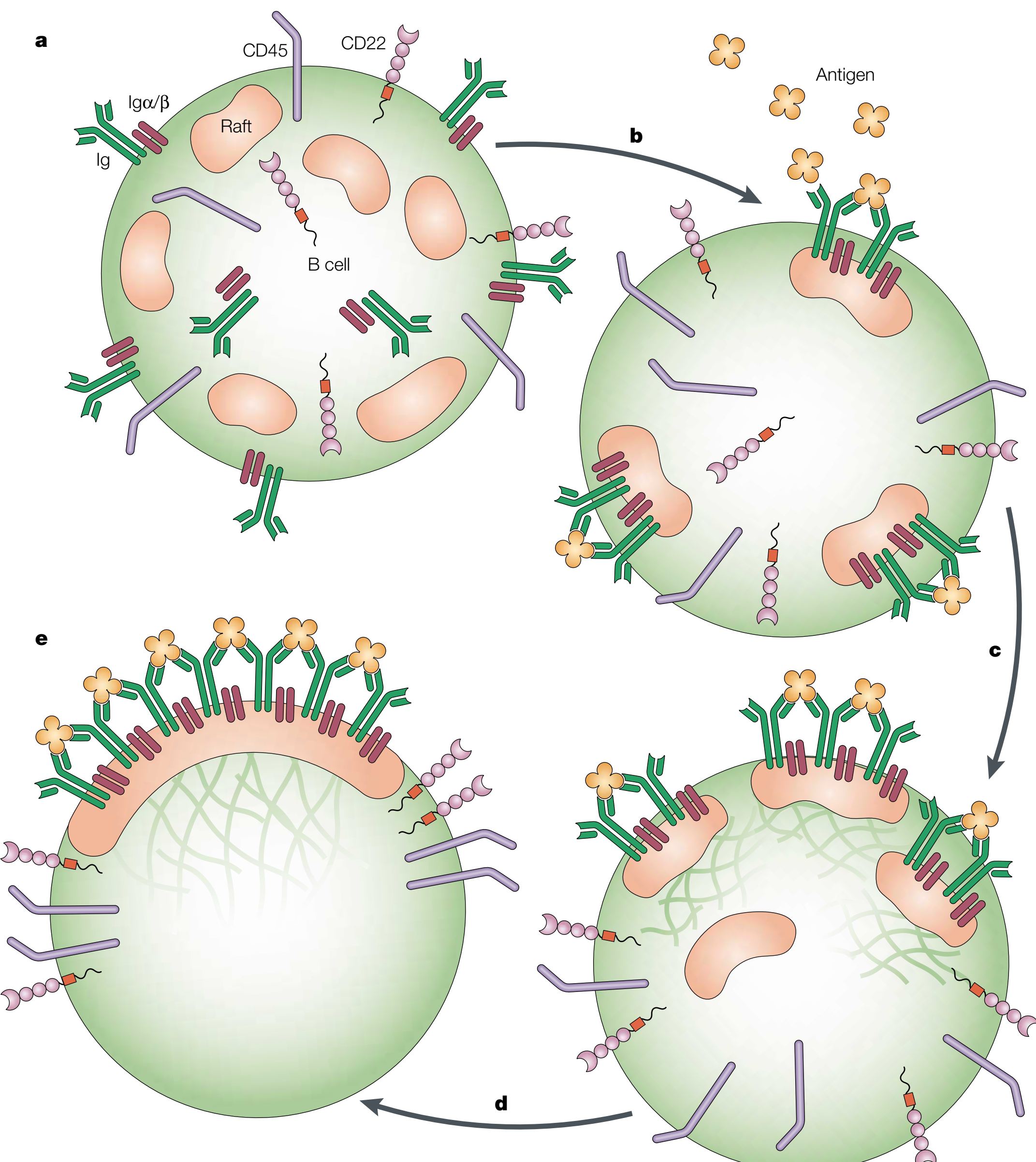

Figure 4 | **Rafts and immune synapses.** In resting B cells, the B-cell receptor (BCR) is excluded from lipid rafts along with other membrane proteins, including CD45 and CD22 (**a**). After antigen binding, the oligomerized BCR associates with rafts by a mechanism that is independent of the actin cytoskeleton and does not require the activity of Lyn (**b**). Signalling is initiated in rafts, leading to the assembly of signalling complexes and association with the actin cytoskeleton. The initiation of signalling promotes raft clustering (**c**). Clustering continues as the clustered rafts are moved to one pole of the cell in a process that probably involves the actin cytoskeleton (**d**). Where antigen is expressed on the surface of another cell, polarization would lead to synapse formation (**e**).

ITIM
Immunoreceptor tyrosine-based inhibitory motif that is present in the cytoplasmic domain of several inhibitory receptors that become tyrosine phosphorylated and recruits the phosphatases SHP1 and SHIP.

GERMINAL CENTRE
A specialized microenvironment in lymph nodes and spleens that forms after antigenic stimulation and is a site of extensive B-cell proliferation and somatic hypermutation of the immunoglobulin genes.

internalization and subsequent processing of antigen bound to the BCR[33,34]. FcγRIIB contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its intracellular domain. When the FcγRIIB is crosslinked, the ITIM tyrosine is phosphorylated by Lyn, resulting in the recruitment of the SH2-domain-containing inositol phosphatase SHIP, which blocks BCR signalling by dephosphorylating phosphatidylinositol triphosphates. In addition, recent evidence indicates that, when FcγRIIB is crosslinked by immune complexes in the absence of antigen binding by the BCR, it signals for apoptosis by a SHIP-independent mechanism[35]. It was suggested that the signalling for apoptosis by the FcγRIIB might have a role in the elimination of B cells in GERMINAL CENTRES that have reduced affinity for antigens as a result of somatic hypermutation. The current evidence indicates that most of the FcγRIIB resides outside lipid rafts in resting B cells, although a proportion seems to be constitutively associated with rafts[36]. The ligation of FcγRIIB and the BCR results in translocation of both receptors into lipid rafts, where the FcγRIIB is phosphorylated and recruits SHIP[36]. So, FcγRIIB seems to act from within rafts to block BCR signalling, and might also block BCR internalization from rafts.

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Preliminary studies indicate that FcγRIIB also translocates into rafts after oligomerization in the absence of antigen binding by the BCR, conditions that result in signalling for apoptosis (S. J. Tzeng and S. K. P., unpublished observations). Interestingly, under these conditions, the subsequent crosslinking of the BCR destabilizes the association of FcγRIIB with rafts, resulting in the exclusion of both FcγRIIB and the BCR from rafts. So, FcγRIIB might function within rafts both to regulate BCR signalling and to signal for apoptosis. The residency of FcγRIIB in rafts for the purpose of signalling for apoptosis might, in turn, be influenced by the BCR.

Relationship between rafts and synapses

T-cell synapse. Studies into the mechanism of T-cell activation after the engagement of peptide–MHC-class-II complexes on antigen-presenting cells (APCs) showed that the ultimate outcome of this interaction is the polarization and clustering of the TCR, co-stimulatory molecules, signalling molecules and cytoskeleton components at the T-cell–APC interface, which has been termed an immunological synapse[37]. Recent evidence indicates that these contact zones are specialized junctions in which the proteins are organized into three-dimensional arrays, the main feature of which is TCRs surrounded by a ring of adhesion molecules. These structures were termed supramolecular activation clusters (SMACs)[38] and contribute to the immunological synapses[39]. There is evidence that the formation of synapses is essential for sustained TCR engagement of peptide–MHC complexes and for T-cell activation[37].

The organization of the synapse raises the question of whether the antigen-presenting B cells similarly organize the peptide–MHC complexes and their adhesion molecules to facilitate the formation of the T-cell synapse. A hint that this might be the case comes from the observations that MHC class II molecules might exist in rafts or, when engaged, be induced to enter rafts, and that the integrity of rafts might be important for the APC function of B cells[40,41]. In the model depicted in FIG. 1, the MHC–peptide complexes that are assembled after BCR-mediated internalization might be ordered on the membrane. Indeed, there is ample evidence that BCR signalling enhances the APC function of B cells independently of their antigen internalization function[4]. It is possible, for example, that BCR signalling influences the association of MHC class II molecules with rafts.

B-cell synapse. Recently, an analogous structure to the immunological synapse has been described using confocal microscopy at the contact site between B cells and cell that express surface antigen[42]. The area of contact between the B cell and the APC was shown to include the BCR, several tyrosine-phosphorylated proteins, actin, phospholipase Cγ2 and GM1, and to exclude CD45, CD22 and the phosphatase SHP1. The BCR was shown to be subsequently internalized from the synapse along with membrane antigen from the APC. Recent studies indicate that, after ligation, the BCR and FcγRIIB both localize to GM1-containing caps on B-cell surfaces along with SHIP, resulting in reduced localization to the caps of several components of the BCR signalling cascade, including Btk, Vav, Rac and F-actin[43].

Earlier studies had established that, after crosslinking by soluble antigens, the BCR forms small patches that coalesce into a cap at one pole of the cell, which is subsequently internalized[44]. The relationship between patching and capping, and the formation of the immunological synapse in B cells is not known. Moreover, it remains to be determined whether the immunological synapse that is observed in B cells has a central role in B-cell signalling, as has been established for the T-cell immunological synapse. Indeed, fundamental differences in the mode of antigen recognition between T cells and B cells indicate that their synapses might not necessarily serve similar functions, despite their similar appearance. However, there does seem to be a correlation between the composition of synapses or caps and the positive or negative outcome of signalling.

Raft-based model for synapse formation. At present, the relationship, if any, between the association of TCR and BCR with rafts and the formation of immunological synapses is not known. This is, in part, because the experimental approaches to the study of synapses and rafts have almost been mutually exclusive. The immunological synapses have been described by microscopy, whereas rafts have been characterized biochemically. Following antigen engagement, the TCR and BCR associate with rafts immediately by a mechanism that seems to be independent of the actin cytoskeleton and Src-family kinase signalling. By contrast, synapse formation for T cells depends on both signalling and the actin cytoskeleton, and takes several minutes[37]. For T cells, synapse formation is initiated by the clustering of TCRs concomitant with a rise in intracellular Ca^{2+} levels. These TCR aggregates are dynamic — forming and reforming over time — but ultimately coalesce into a central cluster[45].

Although there are few data to link rafts and synapses directly, the kinetics of and requirements for the formation of rafts and immunological synapses are consistent with a model in which receptor entry into rafts precedes synapse formation (FIG. 4). For B cells, oligomerization of the BCR results in an increase in affinity of the BCR for lipid rafts and hence to their association with rafts. At this point, the rafts are predicted to be small, submicroscopic domains and the association with receptors is highly dynamic. If the receptor oligomer is sufficiently stable and residency in rafts sufficiently long, signalling is initiated, leading to the assembly of a signalling complex and attachment to the cytoskeleton. The attachment to the actin cytoskeleton and the recruitment of adaptor proteins would cause rafts to cluster and to form more stable receptor-signalling clusters. At this stage, the rafts are predicted to be large enough to be seen by microscopy. Ultimately, the clustered rafts would polarize in an actin-cytoskeleton-dependent process. For B cells, the receptors might accumulate in the region of contact with cells bearing antigen, probably antigen in immune complexes bound to either Fc receptors or complement receptors on a dendritic cell surface.

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Further organization of the signalling and cell-interaction molecules leads to the formation of the immunological synapse by mechanisms that remain to be determined. For B cells, it is not known whether the clustered rafts or synapse are more relevant to prolonged signalling but, for T cells, the immune synapse seems to be essential for substantial signalling.

This model does not necessarily predict that the cells' raft components would be highly concentrated in the synapse, nor that the rafts would continue to function to segregate proteins laterally in the synapse. The lymphocyte membrane is estimated to be composed of ~30% sphingolipids, indicating that rafts compose a large portion of the surface. Only a small proportion of rafts might therefore be involved in any given signalling event. Moreover, a cell's rafts might not be homogeneous and the immune receptors might have an affinity for only a subset of the cells rafts, so only these might cluster at the synapse. Also, it is not known whether the integrity of rafts would be retained in the synapse as the membrane is remodelled to achieve the final, ordered array of receptors. Further studies aimed at elucidating the fate of rafts after receptor activation and the lipid composition of the synapse should help to establish the link between these important organizing structures of immune cell surfaces.

Concluding remarks

The current evidence supports a role for lipid rafts in the initiation and regulation of BCR signalling and antigen trafficking. Rafts seem to function by virtue of their ability to segregate proteins laterally in the plane of the plasma membrane. The description of the relationship between the BCR and rafts focuses attention on several key issues. A fundamental question is the nature of the change induced by BCR oligomerization after antigen binding that results in the association of the BCR with rafts. Another issue is the composition of the rafts themselves and the relationship between raft structure and function in BCR signalling and antigen targeting. Also, the ways that the raft components change during B-cell development (altering the relationship of rafts with the BCR) remain to be determined, as does the relationship of rafts with co-receptors and viral proteins that alter the association of the BCR with rafts.

At present, there are technologies to explore many of the questions about lipid rafts and their components. Indeed, the power of proteomics is already yielding a catalogue of raft components in T cells[46]. It is clear that new technologies to detect protein–protein and protein–lipid interactions, and to monitor the behaviour of individual lipids and proteins in the plasma membranes of living cells, will be important in increasing our understanding of the association of BCR with lipid rafts. This association after antigen binding is a previously unappreciated initial step in B-cell activation. Research focused on elucidating the molecular mechanisms that underlie this association might provide novel strategies to control B-cell activation, both to promote responses in the case of vaccine design and to dampen responses in the case of autoimmune disease.

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