Arch. Immunol. Ther. Exp. (2013) 61:217–228
DOI 10.1007/s00005-013-0221-6

REVIEW

# GSL-Enriched Membrane Microdomains in Innate Immune Responses

**Hitoshi Nakayama · Hideoki Ogawa · Kenji Takamori · Kazuhisa Iwabuchi**

Received: 10 July 2012 / Accepted: 13 February 2013 / Published online: 28 February 2013


**Abstract** Many pathogens target glycosphingolipids (GSLs), which, together with cholesterol, GPI-anchored proteins, and various signaling molecules, cluster on host cell membranes to form GSL-enriched membrane microdomains (lipid rafts). These GSL-enriched membrane microdomains may therefore be involved in host–pathogen interactions. Innate immune responses are triggered by the association of pathogens with phagocytes, such as neutrophils, macrophages and dendritic cells. Phagocytes express a diverse array of pattern-recognition receptors (PRRs), which sense invading microorganisms and trigger pathogen-specific signaling. PRRs can recognize highly conserved pathogen-associated molecular patterns expressed on microorganisms. The GSL lactosylceramide (LacCer, CDw17), which binds to various microorganisms, including *Candida albicans*, is expressed predominantly on the plasma membranes of human mature neutrophils and forms membrane microdomains together with the Src family tyrosine kinase Lyn. These LacCer-enriched membrane microdomains can mediate superoxide generation, migration, and phagocytosis, indicating that LacCer functions as a PRR in innate immunity. Moreover, the interactions of GSL-enriched membrane microdomains with membrane proteins, such as growth factor receptors, are important in mediating the physiological properties of these proteins. Similarly, we recently found that interactions between LacCer-enriched membrane microdomains and CD11b/CD18 (Mac-1, CR3, or αMβ2-integrin) are significant for neutrophil phagocytosis of non-opsonized microorganisms. This review describes the functional role of LacCer-enriched membrane microdomains and their interactions with CD11b/CD18.

**Keywords** Membrane microdomain · Lactosylceramide · Innate immunity · Integrin · Src family kinase

H. Nakayama · K. Iwabuchi
Juntendo University Laboratory for Biochemistry, Faculty of Health Care and Nursing, Chiba, Japan

H. Nakayama · H. Ogawa · K. Takamori · K. Iwabuchi (✉)
Institute for Environmental and Gender-specific Medicine, Juntendo University Graduate School of Medicine, Chiba, Japan
e-mail: iwabuchi@juntendo.ac.jp

## Introduction

Glycosphingolipids (GSLs) are composed of hydrophobic ceramide and hydrophilic sugar moieties (Degroote et al. 2004). More than 400 types of GSL have been identified based on their sugar chain structures (Hakomori 2003). The ceramide structures of GSLs are also highly variable (Kaga et al. 2005). GSL metabolism and composition are specifically altered during the proliferation and differentiation of various cell types (Hakomori 2003; Sonnino et al. 2006), indicating that the expression patterns of GSLs may reflect the functions of these cells. The most important function of GSL, defining cell phenotype, is associated with their clustering with signal transducers to form specialized membrane microdomains (lipid rafts) (Brown and London 1997; Iwabuchi et al. 1998a, b; Yamamura et al. 1997).

GSL-enriched membrane microdomains on various cells interact with the other molecules on plasma membranes to regulate signal transduction. For example, the ganglioside GM3 forms membrane microdomains and associates with insulin receptor in adipocytes (Kabayama et al. 2007). The function of epidermal growth factor receptor is also regulated by its recruitment into GM3-enriched domains (Ringerike et al. 2002; Roepstorff et al. 2002). GM1, the most abundant GSL in brain and neurons, binds to Trk

neurotrophin receptors (Mutoh et al. 1995). GM1-enriched domains have been shown to function as signaling platforms for laminin-1-induced TrkA/β1 integrin-mediated neurite outgrowth (Ichikawa et al. 2009). These findings suggest that the association of GSL-enriched membrane microdomains with receptors regulates receptor-mediated cellular functions.

The innate immune system is one of the most important host defenses against invading microorganisms, such as bacteria, fungi, and viruses. Professional phagocytes, such as neutrophils and macrophages, are the primary cells involved in innate immune responses. These responses are initiated by interactions between pathogen-associated molecular patterns (PAMPs) expressed on microorganisms and pattern-recognition receptors (PRRs) on host cells. Several types of PRR, including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and some GSLs, can directly sense PAMPs without opsonization by C3bi or IgG. Phagocytosis is the most-characterized response in innate immunity against infectious microorganisms. Neutrophils play crucial roles in eliminating microorganisms by phagocytosis and subsequent lysosomal fusion with nascent pathogen-containing vacuolar compartments (phagosomes), forming phagolysosomes (Nauseef 2007).

The binding avidities of microorganisms to several types of GSL (Schengrund 2003) suggest that GSL-enriched membrane microdomains are involved in host–pathogen interactions. Indeed, microorganisms have been shown to recognize and enter host cells via membrane microdomains on these cells (Manes et al. 2003). In human neutrophils, LacCer is highly expressed on plasma membranes (Brackman et al. 1995) and is the most abundant type of GSL (Kniep and Skubitz 1998; Spychalska et al. 2003), forming membrane microdomains with the Src family tyrosine kinase Lyn (Iwabuchi and Nagaoka 2002; Iwabuchi et al. 2008) and acting as a PRR (Iwabuchi and Nagaoka 2002; Nakayama et al. 2008; Sato et al. 2006; Zimmerman et al. 1998). CD11b/CD18 integrin has been shown to collaborate with LacCer-enriched membrane microdomains during neutrophil engulfment of non-opsonized microorganisms (Nakayama et al. 2008). Here we review the association between GSL-enriched membrane microdomains and cell surface receptors and the methods by which these interactions modulate receptor-mediated cell functions. We especially focus on the involvement of LacCer-enriched membrane microdomains in CD11b/CD18 integrin-mediated neutrophil phagocytosis.

## GSL Forms Specialized Membrane Microdomains

The major components of biological membrane are phospholipids, sphingolipids, cholesterol, and membrane-associated proteins. Sphingolipids generally contain long saturated acyl chains (Rietveld and Simons 1998), allowing them to pack tightly together. The ceramide moiety of sphingolipids and the cholesterol sterol-ring system are thought to interact via hydrogen bonds and hydrophobic van der Waar's interactions (Mukherjee and Maxfield 2004). Further *cis* hydrophilic interactions among sphingolipid headgroups promote the lateral associations of sphingolipids and cholesterol. These interactions result in the separation of sphingolipid- and cholesterol-rich membrane microdomains in the cellular membranes. Membrane microdomains, also called lipid rafts, are lipid domains defined by their GSL- and cholesterol-rich nature, enrichment in glycosylphosphatidylinositol (GPI)-anchored proteins and membrane-anchored signaling molecules, and association with the cytoskeleton (Pike 2006). GSLs, composed of hydrophobic ceramide and hydrophilic sugar moieties, can form hydrogen bonds between the hydroxyl groups of sphingosine and acyl amide groups, resulting in *cis* interactions of GSLs within the same membranes (Yoshizaki et al. 2008). In contrast, glycerophospholipids, which have no hydroxyl groups, can only accept hydrogen bonds (Hakomori 2003). GSL self-assembly in GSL-enriched membrane microdomains is not disrupted by cholesterol-binding reagents, such as filipin, or cholesterol-scavenging reagents, such as methyl-β-cyclodextrin and nystatin (Iwabuchi et al. 1998a; Iwabuchi and Nagaoka 2002). These findings suggest that GSL is able to cluster, forming specialized membrane microdomains, in biological membranes.

## GSL-Enriched Membrane Microdomains Affect Integrin Receptor-Mediated Physiological Functions

Numerous studies have demonstrated that membrane microdomains play an important role in a variety of cellular functions, including insulin responses, cell adhesion, neurite outgrowth, polarization, macropinocytosis, low-density lipoprotein uptake, and innate and adaptive immunity (Barbat et al. 2007; Ichikawa et al. 2009; Iwabuchi et al. 1998b; Kabayama et al. 2007; Kiyanagi et al. 2011; Manes et al. 1999; Nakayama et al. 2008; Pierini et al. 2003; Ying et al. 2003). GSL-enriched specialized membrane microdomains have been reported to interact with signaling molecules and membrane proteins to modulate many types of cellular responses.

GM1 is one of the principal gangliosides in the central and peripheral nervous systems. GM1 has been shown to exert positive effects on neuronal growth, differentiation and survival, and to protect against neuronal injury, thus acting as a neurotrophic factor (Favaron et al. 1988; Ferrari et al. 1993). Trk neurotrophin receptors mediate the effects of GM1. Indeed, studies in PC12 cells have shown that exogenous GM1 can stimulate Trk kinase activity, receptor

autophosphorylation and dimerization (Farooqui et al. 1997; Ferrari et al. 1995), and that GM1 can specifically bind to Trk (Mutoh et al. 1995). In neurotumor cells, such as Neuro2a and neuroblastoma cells, the neuritogenic effects of GM1 are thought to be associated with the activation of the non-receptor tyrosine kinase c-Src, which is highly enriched in GSL-enriched membrane microdomains (Prinetti et al. 1999). Recently, GM1 was reported to be involved in laminin-1-induced signaling for neurite outgrowth in PC12 cells and DRG neurons (Ichikawa et al. 2009). The binding of laminin-1 to GM1 induces GM1 aggregation, resulting in the activation of the Src family tyrosine kinase Lyn and promoting the subsequent clustering of TrkA and β1 integrin in GM1-enriched membrane microdomains, thereby stimulating downstream signaling that promotes neurite outgrowth. GM1-enriched membrane microdomains are therefore regarded as signaling platforms for laminin-1-induced TrkA/β1 integrin-mediated neurite outgrowth.

Glycosynapse is a term proposed to describe membrane microdomains involved in carbohydrate-dependent adhesion. The tetraspanins, including CD9, CD81 and CD82, are highly hydrophobic integral membrane proteins, each containing four transmembrane domains, that strongly interact with GSLs (Kawakami et al. 2002). Interestingly, tetraspanins have also been found associated with integrin receptors (Hemler 1998). CD9 colocalizes with the integrin α3 and α5 subunits in glycosynapses. In cell lines expressing high levels of GM3, the association of CD9 and integrin is positively regulated in a GM3-dependent manner (Kawakami et al. 2002; Mitsuzuka et al. 2005). The formation of supramolecular complexes of GM3/CD9/integrin negatively regulates integrin-mediated signal transduction during cell adhesion and tumor cell motility, suggesting that GM3-enriched membrane microdomains are necessary for CD9/integrin-dependent cellular functions.

Another example of the association of GSL-enriched microdomains with integrin is the CD4 ligation-induced aggregation of GM1 and GM3, and the colocalization of these GSLs with lymphocyte function-associated antigen-1 (LFA-1) in T lymphocytes (Trucy et al. 2004). $PI_3$-kinase is needed for the down-regulation of LFA-1-mediated T cell-B cell adhesion. The CD4 ligation-induced down-regulation of LFA-1-dependent adhesion is mediated by the tyrosine kinase p56 Lck-dependent CD4 signaling, which is required for the CD4 ligation-induced down-regulation of LFA-1-dependent adhesion (Mazerolles et al. 1994). p56 Lck is indispensable for the clustering of GM1 and GM3 as well as for the recruitment and association of LFA-1 and $PI_3$-kinase with these gangliosides. Taken together, these findings suggest that the association of GSL-enriched membrane microdomains with integrin receptors is key to the regulation of cell functions mediated by these receptors.

## Membrane Microdomains Collaborate with PRRs in Innate Immune Responses

The innate immune response is a host defense system that senses invading pathogens and activates the production of inflammation-inducing cytokines. Professional phagocytes, such as neutrophils, macrophages and dendritic cells, are responsible for eliminating invading pathogens. The elimination pathway is initiated by the binding of PAMPs to PRRs expressed on phagocytes, forming nascent phagosomes and resulting in the fusion of lysosomes to phagosomes. Following their digestion of pathogens, macrophages and dendritic cells are responsible for MHC-mediated pathogen-derived antigen presentation, resulting in the induction of acquired immune responses. PAMPs are shared by groups of pathogen-related molecules, are essential for pathogen survival, and are not expressed by mammalian cells (Medzhitov and Janeway 2000). PRRs are characterized by their structure, function and localization, and are able to sense a wide range of pathogens, including bacteria, fungi and viruses (Gordon 2002). PAMPs are directly recognized by several types of PRR, such as TLRs (Blander and Medzhitov 2004; Doyle et al. 2004), mannose receptor (Ezekowitz et al. 1990), the lipopolysaccharide (LPS) binding receptor CD14 (Jiang et al. 2005), scavenger receptors (Elomaa et al. 1995; Peiser et al. 2000), the CLR dectin-1 (Herre et al. 2004), and CD11b/CD18 (Brown 1991). PAMPs can be sensed not only by glycoproteins and CLRs, but by GSLs such as LacCer (Iwabuchi and Nagaoka 2002; Iwabuchi et al. 2008; Nakayama et al. 2008; Sato et al. 2006) and GM1 (Naroeni and Porte 2002; Tsai et al. 2003), which constitute specialized membrane microdomains. These types of PRRs act as sensors for non-opsonic infectious microorganisms in host phagocytes. The cooperation between membrane microdomains and PRRs seems to be a key constituent of innate immune responses, playing crucial roles in phagocytosis and activation of inflammation.

TLRs, the first identified PRRs (Akira 2001; Medzhitov and Janeway 2002), are germ-line encoded receptors of the innate immune system that can sense invading pathogens. At least ten TLRs are capable of sensing microbial structures, triggering the production of proinflammatory cytokines. TLRs are expressed on immune cells and can recognize a wide variety of microbial ligands. These include cell components, such as LPS from Gram-negative bacteria and lipoteichoic acid from Gram-positive bacteria, as well as bacterial flagellin, CpG DNA, viral DNA and single stranded RNA (Takeda et al. 2003). TLRs also interact with membrane microdomains. For example, TLR2 and TLR4 were shown to be recruited into membrane microdomains upon stimulation with specific agonists (Nakahira et al. 2006; Soong et al. 2004;

Triantafilou et al. 2006). Recently, the oral/systemic pathogen, *Porphyromonas gingivalis* fimbriae, was reported to induce the association of CXC-chemokine receptor 4 with TLR2 in membrane microdomains, resulting in the inhibition of TLR2-mediated proinflammatory and antimicrobial responses (Hajishengallis et al. 2008). Thus, pathogens may utilize membrane microdomains to mediate molecular associations involved in immune evasion.

The CLR Dectin-1 is critical in recognizing the cell wall component β-glucan, which is expressed on pathogenic fungi, such as *Candida albicans*. Dectin-1 has been reported to mediate the phagocytosis of *C. albicans* and yeast-derived zymosan, inducing the release of inflammatory cytokines (Brown and Gordon 2001; Brown et al. 2002; Kennedy et al. 2007; Taylor et al. 2007). Recently, stimulation of dendritic cells with zymosan was shown to induce the translocation of Dectin-1 into membrane microdomains (Xu et al. 2009), suggesting that the integrity of membrane microdomains is important for signaling. Collectively, these findings suggest that the interactions between PRR and membrane microdomains are ubiquitously crucial events for signal transduction in innate immune responses. To date, however, few studies have focused on the type of GSLs or the mechanisms involved in the interactions between membrane microdomains and PRRs.

## LacCer Acts as a PRR in the Innate Immune System

Various types of pathogens have been shown to bind to carbohydrates of GSLs expressed on host cells (Schengrund 2003). For example, Polyoma virus can bind to the gangliosides GD1a and GT1b in human erythrocytes (Tsai et al. 2003), whereas simian virus 40 (Tsai et al. 2003) and *Brucella suis* (Naroeni and Porte 2002) are recognized by GM1. Not only pathogens themselves, but their toxins, bind to host cells. GM1 is bound specifically by the cholera toxin B subunit (Nagafuku et al. 2012). Globotriaosyl ceramide (Gb3) can bind to Shiga toxin (Stx) B subunit, and acts as a receptor for Stx on the surface of human epithelial and endothelial cells (Lingwood 1996; Louise et al. 1995; Takenouchi et al. 2004). LacCer is also able to bind specifically to various pathogenic microorganisms, including *Escherichia coli*, *Bordetella pertussis*, *Bacillus dysenteriae*, *Propionibacterium freudenreichii*, and *C. albicans* (Sato et al. 2006). *C. albicans*-derived β-glucan (CSBG) binds specifically to LacCer expressed on human neutrophils (Sato et al. 2006). Our results suggested a mechanism by which LacCer recognizes CSBG (Sato et al. 2006). β-Glucans are a heterogeneous group of glucose polymers, consisting of β-1,3-linked β-D-glucopyranosyl units with β-1,6-linked side chains of various distributions and lengths (Liang et al. 1998; Ohno et al. 1999), and are major components of fungal cell walls. CSBG binds to LacCer-, Gb3-, and GalCer-coated culture plates, but not to GlcCer, Gb4, GM1, GM2, or GM3. Galactose is the terminal residue of LacCer, Gb3, and GalCer. The structure of PGG-glucan is similar to that of CSBG, with a β-1,3-glucopyranose glucan backbone containing poly β-1,6 long glucosyl branches (Brown et al. Brown and Gordon 2001). PGG-glucan also binds to GSLs with a terminal galactose residue (Zimmerman et al. 1998). These observations suggest that the galactose residue of LacCer is responsible for the recognition of fungal β-glucans.

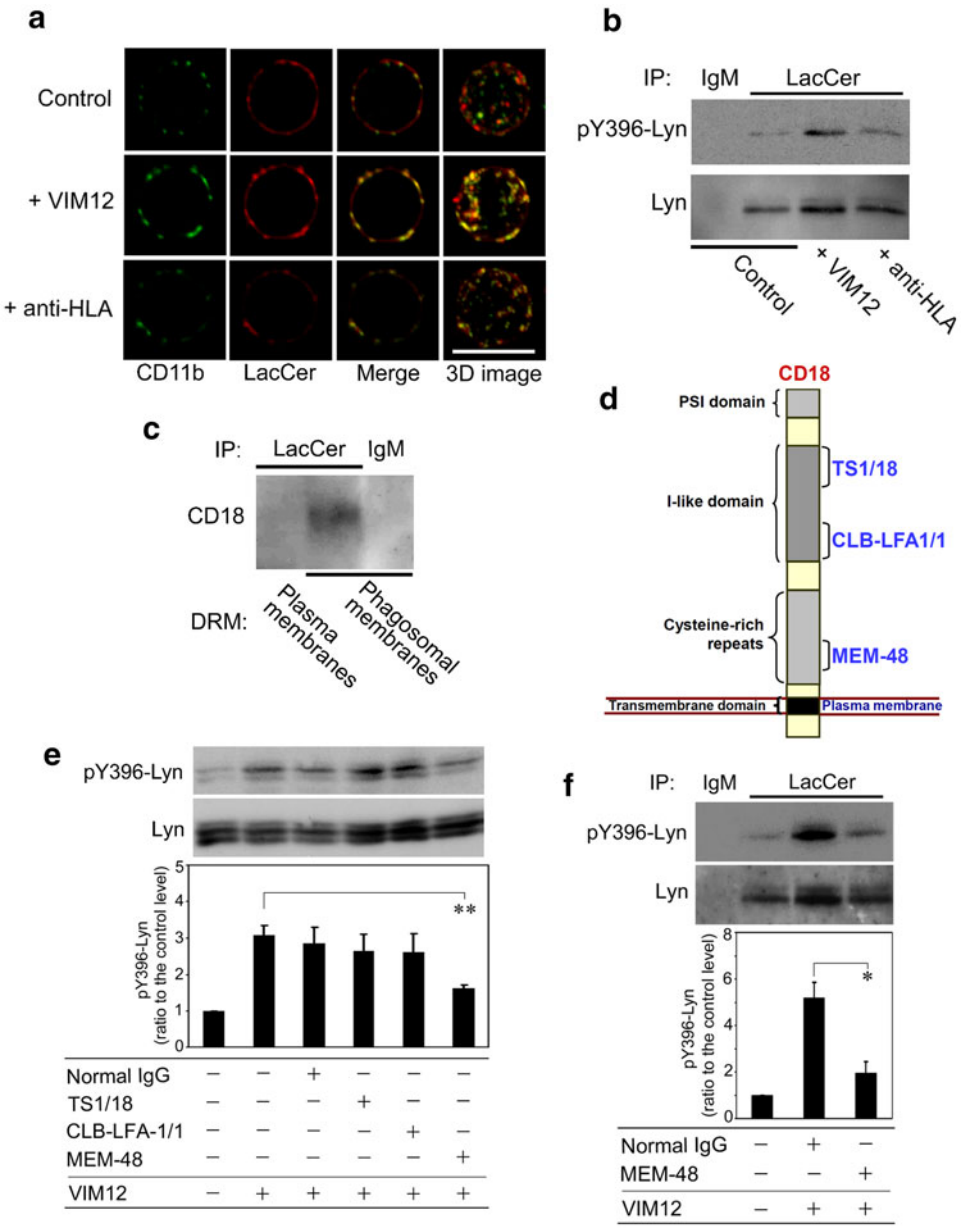
LacCer is highly expressed on the surface of mature neutrophils, constituting more than 70 % of the GSLs on these cells, but is not expressed on myeloblasts or promyelocytes (Brackman et al. 1995; Lund-Johansen et al. 1992; Nakayama et al. 2008). Using immunoelectron microscopy, we recently showed that LacCer is associated with Lyn on plasma membranes (Iwabuchi et al. 2008). LacCer on neutrophil plasma membranes is present expressed as clusters around 45 nm in diameter, with 24 % of these clusters associated with Lyn. Biochemical analysis indicated that LacCer-enriched membrane microdomains can be isolated from detergent-resistant membrane fractions of neutrophils using anti-LacCer antibody and are mainly composed of LacCer, sphingomyelin, phosphatidylcholine, and cholesterol, along with signal transduction molecules (Iwabuchi and Nagaoka 2002). These observations clearly show that LacCer forms Lyn-coupled membrane microdomains on the plasma membranes of human neutrophils.

*Pneumocystis carinii*-derived β-glucan induces rat alveolar epithelial cells to produce significant amounts of MIP-2 via the LacCer expressed on these cells (Hahn et al. 2003). In human neutrophils, CSBG also induces migration, which is completely abolished by LacCer liposomes or the Src family kinase inhibitor PP1. β-Glucan and anti-LacCer antibodies have been shown to induce superoxide production by neutrophils (Iwabuchi and Nagaoka 2002; Lavigne et al. 2007), a response blocked by PP1 (Iwabuchi and Nagaoka 2002). In addition, Lyn molecules in LacCer-enriched membrane microdomains were phosphorylated by anti-LacCer antibodies, CSBG-derived β-glucan (Sato et al. 2006), non-opsonized zymosans, and *Mycobacteria* (unpublished observations). These results suggest that Lyn-coupled LacCer-enriched membrane microdomains are responsible for superoxide production, cell migration, and non-opsonic phagocytosis. Thus, LacCer forms functional membrane microdomains in human neutrophils, acting as a PRR to mediate innate immune responses.

### LacCer-Enriched Membrane Microdomains Interact with CD11b/CD18 in Human Neutrophils

Several neutrophil functions, including adhesion, migration, chemotaxis, phagocytosis, respiratory burst, and degranulation, are regulated by the CD11b/CD18 integrin, also called Mac-1, CR3, and αMβ2-integrin (Arnaout 1990). CD11b/CD18 is closely associated with neutrophil activation at sites of inflammation and infection (Arnaout 1990). In the absence of exposure to inflammatory stimuli, such as chemokines, bacterial products, and cytokines, CD11b/CD18 binds poorly to ligands (Altieri et al. 1988; Detmers et al. 1990; Jones et al. 1998; Weber et al. 1996). Exposure to these stimuli, however, increases the avidity of CD11b and CD18 through a process called "inside-out" signaling (Fagerholm et al. 2006). This inside-out signaling, via specific cytokine or chemoattractant receptors, such as IL-8 receptor, initiates the conversion of CD11b/CD18 from a non-adhesive to an adhesive state, resulting in neutrophil activation (Piccardoni et al. 2004). Ligand binding to CD11b/CD18 delivers Src family kinase-dependent outside-in signals (Piccardoni et al. 2004). Although CD11b/CD18 cytoplasmic regions lack catalytic activity (Dedhar and Hannigan 1996; Rabb et al. 1993), CD11b/CD18 can transduce signals inside the cells. These results suggest that adaptors or mediators are needed for CD11b/CD18-mediated Src family kinase-dependent outside-in signaling. Indeed, the myeloid Src family kinases Hck, Fgr, and Lyn have been reported to be essential for CD11b/CD18-mediated phagocytosis and respiratory burst during *Streptococcus pneumonia* infection (Paul et al. 2008), though the mechanisms connecting integrin-mediated signaling with Src family kinases have not yet been identified. E-selectin (Yago et al. 2010; Zarbock et al. 2008) or P-selectin (Wang et al. 2007) binding to leukocytes also induces the activation of Src family kinases. When neutrophils enter inflamed sites, they tether to and roll on P- and E-selectin expressed on activated endothelial cells, with both selectins binding to P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils. Both CD11b/CD18 and leukocyte-function-associated antigen 1 (LFA1-1, αLβ2-integrin) are involved in the regulation of rolling velocity within inflamed vessels (Zarbock et al. 2007). In particular, αLβ2-integrin plays a critical role in slow rolling, enhancing neutrophil recruitment (Zarbock et al. 2007). The ITAM-containing adaptor proteins DAP12 and Fc receptor-γ are required for E-selectin to induce αLβ2-integrin-mediated slow rolling on intracellular adhesion molecule-1 (ICAM-1). Furthermore, genetic depletion of the Src family kinase Fgr, but not of Hck or Lyn, prevents E-selection-mediated activation of spleen tyrosine kinase Syk and slow rolling on ICAM-1 (Zarbock et al. 2008). Microdomain integrity is crucial to leukocyte rolling on P- and E-selectin (Abbal et al. 2006). For example, microdomain-associated PSGL-1-dependent activation of Syk is necessary for optimal rolling on P-selectin. Recently, E-selectin binding to either PSGL-1 or CD44 was shown to initiate αLβ2-integrin-mediated slow neutrophil rolling through a pathway involving Syk activation by the Src family kinases Fgr, Hck, and Lyn (Yago et al. 2010). Taken together, these findings suggest that Src family kinases and membrane microdomains are key players in αLβ2-integrin activation through the binding of E- and P-selectin to leukocytes. Membrane microdomain components, including Ras family GTPases and Src family kinases, are internalized from the plasma membrane to recycling endosomes during integrin-mediated adhesion and subsequent cell detachment (Norambuena and Schwartz 2011), suggesting the pivotal role of Src family kinases in integrin-mediated adhesion. Src family kinases are also essential for LPS-induced activation of NF-κB and αVβ3-integrin signaling during acute lung injury (Lee et al. 2007). These Src family kinases are therefore among the most important molecules in a diverse array of integrin-mediated cell inflammatory reactions.

The CD11b subunit has a unique structure, with not only a binding site for ligands, such as ICAM-1 and C3bi, but a spatially separated carbohydrate-binding domain. The latter domain serves as a PRR for β-glucan, a major component of fungal cell walls (Thornton et al. 1996; Xia et al. 1999). Binding of β-glucan to CD11b directly induces conformational changes and activation of CD11b, leading to superoxide generation (Vetvicka et al. 1996). We recently showed that, during the phagocytosis of non-opsonized zymosans, CD11b/CD18 and Lyn-coupled LacCer-enriched membrane microdomains accumulate and colocalize in actin-enriched phagocytic cup regions (Nakayama et al. 2008). We also showed that stimulation of CD11b/CD18 with the CD11b-activating antibody VIM12, which binds to β-glucan-recognizable lectin-like domains, induced the formation of large clusters of LacCer and their colocalization with CD11b on plasma membranes in human neutrophils (Fig. 1a). In addition, stimulation with VIM12 induced the phosphorylation of LacCer-associated Lyn (Fig. 1b). These observations suggest that CD11b/CD18-mediated outside-in signaling is dependent on Lyn-coupled LacCer-enriched membrane microdomains. Using anti-LacCer monoclonal antibody (mAb) Huly-m13, we were able to pull down CD18, but not CD11b, from phagosomal membrane microdomains (Fig. 1c), suggesting that CD11b/CD18 is associated with LacCer-enriched membrane microdomains via CD18, with the latter mediating CD11b activation-induced Lyn phosphorylation. We also examined the effects of anti-CD18 mAbs on VIM12-induced Lyn phosphorylation. Anti-CD18 mAb TS1/18 recognizes an epitope localized to residues 123–163 in the




N-terminal portion and residues 303–344 in the C-terminal portion of the conserved domain (Huang et al. 2000) (Fig. 1d). The anti-CD18 mAbs CLB-LFA-1/1 and MEM-48 recognize epitopes localized to the cysteine-rich repeat 3 domains (residues 303–344) (Huang et al. 2000), and residues 514–553 in the C-terminal portion of the conserved domain (Lu et al. 2001), respectively (Fig. 1d). Neutrophils were treated with these anti-CD18 antibodies, followed by activation with VIM12. Among these CD18 mAbs, only MEM-48 inhibited VIM12-induced Lyn phosphorylation in neutrophils (Fig. 1e), as well as significantly inhibiting VIM12-induced LacCer-associated Lyn phosphorylation (Fig. 1f). These results indicate that CD18 is involved in CD11b activation-induced Lyn phosphorylation in LacCer-enriched membrane microdomains. Collectively, these findings show that the anti-CD18 mAb MEM-48, which recognizes the extracellular juxtamembrane region of CD18, inhibits the VIM12-induced phosphorylation of Lyn in LacCer-enriched membrane microdomains. MEM-48 also significantly inhibited neutrophil adhesion to immobilized zymosan-derived β-glucan (Lavigne et al. 2006), which can be recognized by both CD11b and LacCer (Vetvicka et al. 1996; Zimmerman et al. 1998). A schematic image of the mechanism involved in LacCer-enriched membrane microdomain-mediated CD11b/CD18-dependent neutrophil phagocytosis is shown in Fig. 2. Similar to the interaction of GM3 with insulin receptor or epidermal growth factor receptor, LacCer

◀ **Fig. 1** Ligand binding to CD11b induces activation signals through the interaction between CD18 and LacCer-enriched membrane microdomains. **a** VIM12-induced colocalization of CD11b and LacCer. Neutrophils were incubated in the presence or absence of anti-CD11b activating $F(ab')_2$ VIM12 or anti-HLA class I antigen $F(ab')_2$ for 5 min at 37 °C. After crosslinking, the cells were fixed and incubated with anti-CD11b mAb M1/70 (*green*) and Alexa546-conjugated anti-LacCer mAb T5A7 (*red*), and stained with Alexa488-conjugated donkey anti-rat secondary IgG. Control, no crosslinking; Anti-CD11b, crosslinking of CD11b; Anti-HLA, crosslinking of HLA. DIC, differential interference contrast image. White bar, 10 μm. 3D image, three-dimensional image. **b** VIM12 induced activation of Lyn in LacCer-enriched membrane microdomains. Neutrophils were crosslinked with anti-CD11b $F(ab')_2$ VIM12 or anti-HLA class I antigen $F(ab')_2$ for 5 min at 37 °C. To determine whether LacCer-associated Lyn was activated by VIM12, the crosslinked neutrophils were lysed, and the lysates were incubated with anti-LacCer mAb Huly-m13 or normal mouse IgM. The immunoprecipitates were subjected to SDS-PAGE and immunoblotted sequentially with anti-pY396 Lyn IgG and anti-Lyn IgG. IP, immunoprecipitation. **c** Immunoprecipitation of LacCer binding proteins from plasma membrane and phagosomal membrane detergent-resistant microdomains (DRM). Plasma and phagosomal membrane DRMs of neutrophils were incubated with anti-LacCer mAb Huly-m13. The immunoprecipitates were subjected to SDS-PAGE/immunoblotting with anti-CD18 mAb MEM-48. IP, immunoprecipitation. **d** CD18 subdomains recognized by clones of anti-CD18 mAb. PSI domain, N-terminal cysteine-rich plexin/semaphorin/integrin domain. **e** Effects of anti-CD18 mAbs on VIM12-induced Lyn activation. Neutrophils were incubated for 30 min on ice without (untreated) or with 10 μg of anti-CD18 mAbs (TS1/18, CLB-LFA-1/1, and MEM-48) or normal mouse IgG. The neutrophils were crosslinked with anti-CD11b $F(ab')_2$ VIM12 for 5 min at 37 °C, and Lyn activation was analyzed by sequential immunoblotting with anti-pY396 Lyn IgG and anti-Lyn IgG. The ratio of band intensities of activated Lyn to total Lyn in each lane was calculated. Data are presented as ratios relative to control (without antibodies), and are expressed as the mean ± SD of three independent experiments. $^{**}p < 0.01$ compared with the untreated group. **f** Effects of the anti-CD18 mAb MEM-48 on VIM12-induced Lyn activation in LacCer-enriched membrane microdomains. Neutrophils were incubated for 30 min on ice in the presence of 10 μg of anti-CD18 mAb MEM-48 or normal mouse IgG, followed by crosslinking with anti-CD11b $F(ab')_2$ VIM12 for 5 min at 37 °C. The cells were lysed, the lysates were incubated with anti-LacCer mAb Huly-m13 or normal mouse IgM, and the immunoprecipitates were subjected to SDS-PAGE and sequentially immunoblotted with anti-pY396 Lyn IgG and anti-Lyn IgG. Data are presented as ratios relative to control (without antibodies) and are expressed as the mean ± SD of three independent experiments. $^{*}p < 0.05$ compared with the normal mouse IgG-treated group. *IP* immunoprecipitation

seems to interact with CD11b/CD18 via the proximal membrane region of CD18 on the neutrophil plasma membrane. The GPI-anchored neutrophil-specific receptor NB1 (CD177), which presents the autoantigen proteinase 3 (PR3) on the plasma membranes of neutrophils, colocalizes with CD11b/CD18 within membrane microdomains (Jerke et al. 2011), suggesting the involvement of NB1-CD11b/CD18 interactions in PR3-ANCA (anti-neutrophil cytoplasmic auto-antibodies)-mediated neutrophil activation. Thus, some adaptors may be required for interactions between LacCer-enriched membrane microdomains and CD18, although the molecular basis for these interactions remains to be determined.

## LacCer-Enriched Membrane Microdomain-Mediated CD11b/CD18-Dependent Phagocytosis and Intracellular Parasites

Intracellular parasites, such as *Listeria*, *Salmonella*, and *Mycobacteria*, reside inside host cells by manipulating various innate immune responses. Intracellular parasites engulfed by phagocytes under non-opsonized conditions utilize host membrane microdomains (Manes et al. 2003). Once these parasites are internalized within the phagosome, they must avoid degradation and activation of MHC-mediated antigen presentation by escaping from the vacuolar compartment to reach the cytosol, or by arresting the fusion of lysosomes with phagosomes (Baorto et al. 1997; Brumell et al. 2003; Gatfield and Pieters 2000; Gekara and Weiss 2004; Harrison et al. 2004; Knodler et al. 2003; Stuart et al. 2003; Tamilselvam and Daefler 2008; Watarai et al. 2002). *Listeria monocytogenes* disrupts the vacuolar membrane by secreting a pore-forming cytolysin, listeriolysin, which has been shown to interact with membrane microdomains (Gekara and Weiss 2004), resulting in an escape from the vacuolar compartment to the cytoplasm. Some microorganisms utilize membrane microdomains to prevent fusion with lysosomes. For example, *Salmonella*-containing vacuoles (SCVs) do not mature into phagolysosomes but rather induce the formation of an intracellular filament network, called *Salmonella*-induced filaments (Sif) (Brumell et al. 2003; Harrison et al. 2004). The *Salmonella enterica* effector protein PipB2 targets the membrane microdomains of SCVs and Sifs (Knodler et al. 2003). Some mycobacterial species, including the human pathogen *Mycobacterium tuberculosis*, also use membrane microdomains to enter cells and to prevent the fusion of lysosomes with bacteria-containing phagosomes, resulting in escape from degradation by phagocytes. Coronin-1, also called tryptophan aspartate containing coat protein, is a membrane microdomain-associated protein reported to be involved in the escape mechanisms of pathogenic mycobacteria (Gatfield and Pieters 2000). These observations suggest that prevention of membrane microdomain-dependent phagocytosis of non-opsonized intracellular pathogens may inhibit the escape of pathogens from killing by phagocytes. However, little is known about the membrane microdomain-associated molecules that participate in the phagocytosis of these non-opsonized intracellular pathogens. LacCer-enriched membrane microdomains are essential for phagocytosis of CD11b/CD18-mediated non-opsonized microorganisms by human neutrophils

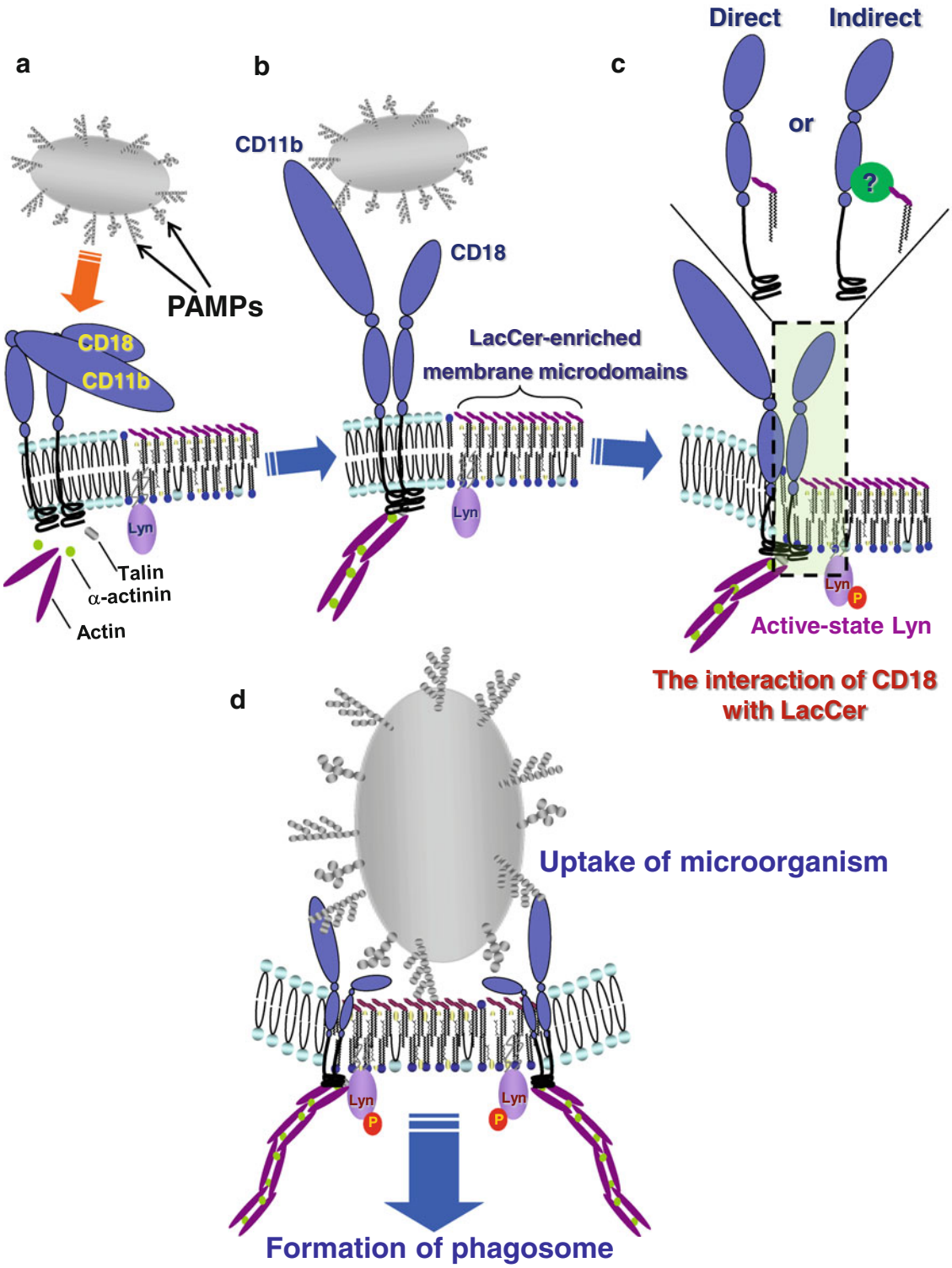



**Fig. 2** LacCer-enriched membrane microdomains mediate CD11b/CD18-dependent neutrophil phagocytosis. Upon binding to CD11b/CD18 of PAMPs expressed on pathogens, such as β-glucan (**a**), CD11b/CD18 is activated and undergoes a conformational change, resulting in the rearrangement of cytoskeletal proteins, such as talin and α-actinin (**b**). The CD11b/CD18 subsequently translocates into LacCer-enriched membrane microdomains, allowing CD11b/CD18 to transmit stimulatory signals to Lyn through the interaction of LacCer with residues 514–553 in the C-terminal portion of the conserved domain of CD18, although it is unclear whether this interaction is direct or indirect (**c**). "?" indicates an adaptor molecule. These signaling cascades lead to the formation of actin-enriched phagocytic cups, resulting in phagosome formation (**d**)

(Nakayama et al. 2008). Membrane microdomains and CD11b/CD18 were shown to be involved in the phagocytosis of non-opsonized mycobacteria by human neutrophils (Peyron et al. 2000). Moreover, our unpublished observations indicated that both LacCer-enriched membrane microdomains and CD11b/CD18 are necessary for the phagocytosis of non-opsonized *Mycobacteria* in human neutrophils. Taken together, these findings suggest that LacCer-enriched membrane microdomain-mediated CD11b/CD18-dependent phagocytosis of pathogenic mycobacteria may be related to mechanisms of escape from host degradation pathways responsible for preventing lysosomal fusion with phagosomes.

## Concluding Remarks

We have reviewed the mechanisms by which GSL-enriched membrane microdomains are related to physiological functions in different types of cells. Our most recent data revealed that a new type of glycolipid, phosphatidyl glucoside (PtdGlc), forms lipid domains functionally distinct from LacCer-enriched membrane microdomains, and that these PtdGlc-enriched domains interact with Fas (CD95) on plasma membranes to mediate neutrophil apoptosis (Kina et al. 2011). These findings indicate that various types of lipid domains coexist on plasma membranes, even within the same cell type, and transduce signals distinct from each other. Thus, interactions between GSL-enriched membrane microdomains and their partner molecules may be ubiquitously important for transducing specific signals inside cells. Greater understanding of the mechanism of association between GSL-enriched membrane microdomains and partner molecules in different aspects of pathogenesis may provide novel therapeutic targets for many types of diseases.

This review has focused on the functional role of LacCer-enriched membrane microdomains in innate immune responses and the significance of interactions between LacCer-enriched membrane microdomains and CD11b/CD18. We recently showed that LacCer is expressed not only on neutrophils, but on human monocyte-derived dendritic cells (Kina et al. 2011). In addition, *Streptococcus suis* capsular polysaccharide was shown to prevent LacCer-dependent recognition by murine macrophages (Houde et al. 2012). These findings suggest that LacCer-enriched membrane microdomain-mediated CD11b/CD18-dependent phagocytosis by professional phagocytes is extensively utilized in innate immune responses, and is a crucial event in the first line of defense against invading pathogens. It is not yet known, however, whether LacCer can directly interact with CD18 without adaptors. Many types of intracellular parasites have different mechanisms of pathogenesis and diverse clinical presentations, despite having a common feature, i.e., the molecular complex of PRRs and membrane microdomains. Therefore, the elucidation of the structural and molecular mechanisms underlying the interactions of GSL-enriched membrane microdomains with PRRs in immune system cells will provide seeds for the development of new pharmacological reagents to treat many types of infectious diseases.

**Acknowledgments** This work was supported in part by a Grant-in-aid (S0991013) from the Foundation of Strategic Research Projects in Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

## References

Abbal C, Lambelet M, Bertaggia D et al (2006) Lipid raft adhesion receptors and Syk regulate selectin-dependent rolling under flow conditions. Blood 108:3352–3359

Akira S (2001) Toll-like receptors and innate immunity. Adv Immunol 78:1–56

Altieri DC, Bader R, Mannucci PM et al (1988) Oligospecificity of the cellular adhesion receptor Mac-1 encompasses an inducible recognition specificity for fibrinogen. J Cell Biol 107:1893–1900

Arnaout MA (1990) Structure and function of the leukocyte adhesion molecules CD11/CD18. Blood 75:1037–1050

Baorto DM, Gao Z, Malaviya R et al (1997) Survival of FimH-expressing enterobacteria in macrophages relies on glycolipid traffic. Nature 389:636–639

Barbat C, Trucy M, Sorice M et al (2007) p56lck, LFA-1 and PI3K but not SHP-2 interact with GM1- or GM3-enriched microdomains in a CD4-p56lck association-dependent manner. Biochem J 402:471–481

Blander JM, Medzhitov R (2004) Regulation of phagosome maturation by signals from toll-like receptors. Science 304:1014–1018

Brackman D, Lund-Johansen F, Aarskog D (1995) Expression of leukocyte differentiation antigens during the differentiation of HL-60 cells induced by 1,25-dihydroxyvitamin D3: comparison with the maturation of normal monocytic and granulocytic bone marrow cells. J Leukoc Biol 58:547–555

Brown EJ (1991) Complement receptors and phagocytosis. Curr Opin Immunol 3:76–82

Brown GD, Gordon S (2001) Immune recognition. A new receptor for beta-glucans. Nature 413:36–37

Brown DA, London E (1997) Structure of detergent-resistant membrane domains: does phase separation occur in biological membranes? Biochem Biophys Res Commun 240:1–7

Brown GD, Taylor PR, Reid DM et al (2002) Dectin-1 is a major beta-glucan receptor on macrophages. J Exp Med 196:407–412

Brumell JH, Kujat-Choy S, Brown NF et al (2003) SopD2 is a novel type III secreted effector of Salmonella typhimurium that targets late endocytic compartments upon delivery into host cells. Traffic 4:36–48

Dedhar S, Hannigan GE (1996) Integrin cytoplasmic interactions and bidirectional transmembrane signalling. Curr Opin Cell Biol 8:657–669

Degroote S, Wolthoorn J, van Meer G (2004) The cell biology of glycosphingolipids. Semin Cell Dev Biol 15:375–387

Detmers PA, Lo SK, Olsen-Egbert E et al (1990) Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. J Exp Med 171:1155–1162

Doyle SE, O'Connell RM, Miranda GA et al (2004) Toll-like receptors induce a phagocytic gene program through p38. J Exp Med 199:81–90

Elomaa O, Kangas M, Sahlberg C et al (1995) Cloning of a novel bacteria-binding receptor structurally related to scavenger receptors and expressed in a subset of macrophages. Cell 80:603–609

Ezekowitz RA, Sastry K, Bailly P et al (1990) Molecular characterization of the human macrophage mannose receptor: demonstration of multiple carbohydrate recognition-like domains and phagocytosis of yeasts in Cos-1 cells. J Exp Med 172:1785–1794

Fagerholm SC, Varis M, Stefanidakis M et al (2006) alpha-Chain phosphorylation of the human leukocyte CD11b/CD18 (Mac-1) integrin is pivotal for integrin activation to bind ICAMs and leukocyte extravasation. Blood 108:3379–3386

Farooqui T, Franklin T, Pearl DK et al (1997) Ganglioside GM1 enhances induction by nerve growth factor of a putative dimer of TrkA. J Neurochem 68:2348–2355

Favaron M, Manev H, Alho H et al (1988) Gangliosides prevent glutamate and kainate neurotoxicity in primary neuronal cultures of neonatal rat cerebellum and cortex. Proc Natl Acad Sci USA 85:7351–7355

Ferrari G, Batistatou A, Greene LA (1993) Gangliosides rescue neuronal cells from death after trophic factor deprivation. J Neurosci 13:1879–1887

Ferrari G, Anderson BL, Stephens RM et al (1995) Prevention of apoptotic neuronal death by GM1 ganglioside. Involvement of Trk neurotrophin receptors. J Biol Chem 270:3074–3080

Gatfield J, Pieters J (2000) Essential role for cholesterol in entry of mycobacteria into macrophages. Science 288:1647–1650

Gekara NO, Weiss S (2004) Lipid rafts clustering and signalling by listeriolysin O. Biochem Soc Trans 32:712–714

Gordon S (2002) Pattern recognition receptors: doubling up for the innate immune response. Cell 111:927–930

Hahn PY, Evans SE, Kottom TJ et al (2003) Pneumocystis carinii cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. J Biol Chem 278:2043–2050

Hajishengallis G, Wang M, Liang S et al (2008) Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function. Proc Natl Acad Sci USA 105:13532–13537

Hakomori S (2003) Structure, organization, and function of glycosphingolipids in membrane. Curr Opin Hematol 10:16–24

Harrison RE, Brumell JH, Khandani A et al (2004) Salmonella impairs RILP recruitment to Rab7 during maturation of invasion vacuoles. Mol Biol Cell 15:3146–3154

Hemler ME (1998) Integrin associated proteins. Curr Opin Cell Biol 10:578–585

Herre J, Marshall AS, Caron E et al (2004) Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. Blood 104:4038–4045

Houde M, Gottschalk M, Gagnon F et al (2012) Streptococcus suis capsular polysaccharide inhibits phagocytosis through destabilization of lipid microdomains and prevents lactosylceramide-dependent recognition. Infect Immun 80:506–517

Huang C, Zang Q, Takagi J et al (2000) Structural and functional studies with antibodies to the integrin beta 2 subunit. A model for the I-like domain. J Biol Chem 275:21514–21524

Ichikawa N, Iwabuchi K, Kurihara H et al (2009) Binding of laminin-1 to monosialoganglioside GM1 in lipid rafts is crucial for neurite outgrowth. J Cell Sci 122:289–299

Iwabuchi K, Nagaoka I (2002) Lactosylceramide-enriched glycosphingolipid signaling domain mediates superoxide generation from human neutrophils. Blood 100:1454–1464

Iwabuchi K, Handa K, Hakomori S (1998a) Separation of "glycosphingolipid signaling domain" from caveolin-containing membrane fraction in mouse melanoma B16 cells and its role in cell adhesion coupled with signaling. J Biol Chem 273: 33766–33773

Iwabuchi K, Yamamura S, Prinetti A et al (1998b) GM3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate–carbohydrate interaction in mouse melanoma B16 cells. J Biol Chem 273:9130–9138

Iwabuchi K, Prinetti A, Sonnino S et al (2008) Involvement of very long fatty acid-containing lactosylceramide in lactosylceramide-mediated superoxide generation and migration in neutrophils. Glycoconj J 25:357–374

Jerke U, Rolle S, Dittmar G et al (2011) Complement receptor Mac-1 is an adaptor for NB1 (CD177)-mediated PR3-ANCA neutrophil activation. J Biol Chem 286:7070–7081

Jiang Z, Georgel P, Du X et al (2005) CD14 is required for MyD88-independent LPS signaling. Nat Immunol 6:565–570

Jones SL, Knaus UG, Bokoch GM et al (1998) Two signaling mechanisms for activation of alphaM beta2 avidity in polymorphonuclear neutrophils. J Biol Chem 273:10556–10566

Kabayama K, Sato T, Saito K et al (2007) Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc Natl Acad Sci USA 104: 13678–13683

Kaga N, Kazuno S, Taka H et al (2005) Isolation and mass spectrometry characterization of molecular species of lactosylceramides using liquid chromatography-electrospray ion trap mass spectrometry. Anal Biochem 337:316–324

Kawakami Y, Kawakami K, Steelant WF et al (2002) Tetraspanin CD9 is a "proteolipid", and its interaction with alpha 3 integrin in microdomain is promoted by GM3 ganglioside, leading to inhibition of laminin-5-dependent cell motility. J Biol Chem 277:34349–34358

Kennedy AD, Willment JA, Dorward DW et al (2007) Dectin-1 promotes fungicidal activity of human neutrophils. Eur J Immunol 37:467–478

Kina K, Masuda H, Nakayama H et al (2011) The novel neutrophil differentiation marker phosphatidylglucoside mediates neutrophil apoptosis. J Immunol 186:5323–5332

Kiyanagi T, Iwabuchi K, Shimada K et al (2011) Involvement of cholesterol-enriched microdomains in class A scavenger receptor-mediated responses in human macrophages. Atherosclerosis 215:60–69

Kniep B, Skubitz KM (1998) Subcellular localization of glycosphingolipids in human neutrophils. J Leukoc Biol 63:83–88

Knodler LA, Vallance BA, Hensel M et al (2003) Salmonella type III effectors PipB and PipB2 are targeted to detergent-resistant microdomains on internal host cell membranes. Mol Microbiol 49:685–704

Lavigne LM, Albina JE, Reichner JS (2006) Beta-glucan is a fungal determinant for adhesion-dependent human neutrophil functions. J Immunol 177:8667–8675

Lavigne LM, O'Brien XM, Kim M et al (2007) Integrin engagement mediates the human polymorphonuclear leukocyte response to a fungal pathogen-associated molecular pattern. J Immunol 178: 7276–7282

Lee HS, Moon C, Lee HW et al (2007) Src tyrosine kinases mediate activations of NF-kappaB and integrin signal during lipopolysaccharide-induced acute lung injury. J Immunol 179:7001–7011

Liang J, Melican D, Cafro L et al (1998) Enhanced clearance of a multiple antibiotic resistant Staphylococcus aureus in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Int J Immunopharmacol 20:595–614

Lingwood CA (1996) Role of verotoxin receptors in pathogenesis. Trends Microbiol 4:147–153

Louise CB, Kaye SA, Boyd B et al (1995) Shiga toxin-associated hemolytic uremic syndrome: effect of sodium butyrate on sensitivity of human umbilical vein endothelial cells to Shiga toxin. Infect Immun 63:2766–2769

Lu C, Ferzly M, Takagi J et al (2001) Epitope mapping of antibodies to the C-terminal region of the integrin beta 2 subunit reveals regions that become exposed upon receptor activation. J Immunol 166:5629–5637

Lund-Johansen F, Olweus J, Horejsi V et al (1992) Activation of human phagocytes through carbohydrate antigens (CD15, sialyl-CD15, CDw17, and CDw65). J Immunol 148:3221–3229

Manes S, Mira E, Gomez-Mouton C et al (1999) Membrane raft microdomains mediate front-rear polarity in migrating cells. EMBO J 18:6211–6220

Manes S, del Real G, Martinez AC (2003) Pathogens: raft hijackers. Nat Rev Immunol 3:557–568

Mazerolles F, Barbat C, Meloche S et al (1994) LFA-1-mediated antigen-independent T cell adhesion is regulated by CD4 and p56lck tyrosine kinase. J Immunol 152:5670–5679

Medzhitov R, Janeway C Jr (2000) Innate immune recognition: mechanisms and pathways. Immunol Rev 173:89–97

Medzhitov R, Janeway CA Jr (2002) Decoding the patterns of self and nonself by the innate immune system. Science 296:298–300

Mitsuzuka K, Handa K, Satoh M et al (2005) A specific microdomain ("glycosynapse 3") controls phenotypic conversion and reversion of bladder cancer cells through GM3-mediated interaction of alpha3beta1 integrin with CD9. J Biol Chem 280: 35545–35553

Mukherjee S, Maxfield FR (2004) Membrane domains. Annu Rev Cell Dev Biol 20:839–866

Mutoh T, Tokuda A, Miyadai T et al (1995) Ganglioside GM1 binds to the Trk protein and regulates receptor function. Proc Natl Acad Sci USA 92:5087–5091

Nagafuku M, Okuyama K, Onimaru Y et al (2012) CD4 and CD8 T cells require different membrane gangliosides for activation. Proc Natl Acad Sci USA 109:E336–E342

Nakahira K, Kim HP, Geng XH et al (2006) Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. J Exp Med 203:2377–2389

Nakayama H, Yoshizaki F, Prinetti A et al (2008) Lyn-coupled LacCer-enriched lipid rafts are required for CD11b/CD18-mediated neutrophil phagocytosis of nonopsonized microorganisms. J Leukoc Biol 83:728–741

Naroeni A, Porte F (2002) Role of cholesterol and the ganglioside GM(1) in entry and short-term survival of Brucella suis in murine macrophages. Infect Immun 70:1640–1644

Nauseef WM (2007) How human neutrophils kill and degrade microbes: an integrated view. Immunol Rev 219:88–102

Norambuena A, Schwartz MA (2011) Effects of integrin-mediated cell adhesion on plasma membrane lipid raft components and signaling. Mol Biol Cell 22:3456–3464

Ohno N, Uchiyama M, Tsuzuki A et al (1999) Solubilization of yeast cell-wall beta-(1–>3)-D-glucan by sodium hypochlorite oxidation and dimethyl sulfoxide extraction. Carbohydr Res 316: 161–172

Paul R, Obermaier B, Van Ziffle J et al (2008) Myeloid Src kinases regulate phagocytosis and oxidative burst in pneumococcal meningitis by activating NADPH oxidase. J Leukoc Biol 84:1141–1150

Peiser L, Gough PJ, Kodama T et al (2000) Macrophage class A scavenger receptor-mediated phagocytosis of Escherichia coli: role of cell heterogeneity, microbial strain, and culture conditions in vitro. Infect Immun 68:1953–1963

Peyron P, Bordier C, N'Diaye EN et al (2000) Nonopsonic phagocytosis of Mycobacterium kansasii by human neutrophils depends on cholesterol and is mediated by CR3 associated with glycosylphosphatidylinositol-anchored proteins. J Immunol 165: 5186–5191

Piccardoni P, Manarini S, Federico L et al (2004) SRC-dependent outside-in signalling is a key step in the process of autoregulation of beta2 integrins in polymorphonuclear cells. Biochem J 380:57–65

Pierini LM, Eddy RJ, Fuortes M et al (2003) Membrane lipid organization is critical for human neutrophil polarization. J Biol Chem 278:10831–10841

Pike LJ (2006) Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. J Lipid Res 47:1597–1598

Prinetti A, Iwabuchi K, Hakomori S (1999) Glycosphingolipid-enriched signaling domain in mouse neuroblastoma Neuro2a cells. Mechanism of ganglioside-dependent neuritogenesis. J Biol Chem 274:20916–20924

Rabb H, Michishita M, Sharma CP et al (1993) Cytoplasmic tails of human complement receptor type 3 (CR3, CD11b/CD18) regulate ligand avidity and the internalization of occupied receptors. J Immunol 151:990–1002

Rietveld A, Simons K (1998) The differential miscibility of lipids as the basis for the formation of functional membrane rafts. Biochim Biophys Acta 1376:467–479

Ringerike T, Blystad FD, Levy FO et al (2002) Cholesterol is important in control of EGF receptor kinase activity but EGF receptors are not concentrated in caveolae. J Cell Sci 115:1331–1340

Roepstorff K, Thomsen P, Sandvig K et al (2002) Sequestration of epidermal growth factor receptors in non-caveolar lipid rafts inhibits ligand binding. J Biol Chem 277:18954–18960

Sato T, Iwabuchi K, Nagaoka I et al (2006) Induction of human neutrophil chemotaxis by Candida albicans-derived beta-1,6-long glycoside side-chain-branched beta-glucan. J Leukoc Biol 80:204–211

Schengrund CL (2003) "Multivalent" saccharides: development of new approaches for inhibiting the effects of glycosphingolipid-binding pathogens. Biochem Pharmacol 65:699–707

Sonnino S, Prinetti A, Mauri L et al (2006) Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains. Chem Rev 106:2111–2125

Soong G, Reddy B, Sokol S et al (2004) TLR2 is mobilized into an apical lipid raft receptor complex to signal infection in airway epithelial cells. J Clin Invest 113:1482–1489

Spychalska J, Smolenska-Sym G, Zdebska E et al (2003) Quantitative analysis of LacCer/CDw17 in human myelogenous leukaemic cells. Cell Mol Biol Lett 8:911–917

Stuart ES, Webley WC, Norkin LC (2003) Lipid rafts, caveolae, caveolin-1, and entry by Chlamydiae into host cells. Exp Cell Res 287:67–78

Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21:335–376

Takenouchi H, Kiyokawa N, Taguchi T et al (2004) Shiga toxin binding to globotriaosyl ceramide induces intracellular signals that mediate cytoskeleton remodeling in human renal carcinoma-derived cells. J Cell Sci 117:3911–3922

Tamilselvam B, Daefler S (2008) Francisella targets cholesterol-rich host cell membrane domains for entry into macrophages. J Immunol 180:8262–8271

Taylor PR, Tsoni SV, Willment JA et al (2007) Dectin-1 is required for beta-glucan recognition and control of fungal infection. Nat Immunol 8:31–38

Thornton BP, Vetvicka V, Pitman M et al (1996) Analysis of the sugar specificity and molecular location of the beta-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). J Immunol 156:1235–1246

Triantafilou M, Gamper FG, Haston RM et al (2006) Membrane sorting of Toll-like receptor (TLR)-2/6 and TLR2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting. J Biol Chem 281:31002–31011

Trucy M, Barbat C, Sorice M et al (2004) CD4-induced down-regulation of T cell adhesion to B cells is associated with localization of phosphatidyl inositol 3-kinase and LFA-1 in distinct membrane domains. Eur J Immunol 34:2168–2178

Tsai B, Gilbert JM, Stehle T et al (2003) Gangliosides are receptors for murine polyoma virus and SV40. EMBO J 22:4346–4355

Vetvicka V, Thornton BP, Ross GD (1996) Soluble beta-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J Clin Invest 98:50–61

Wang HB, Wang JT, Zhang L et al (2007) P-selectin primes leukocyte integrin activation during inflammation. Nat Immunol 8:882–892

Watarai M, Makino S, Fujii Y et al (2002) Modulation of Brucella-induced macropinocytosis by lipid rafts mediates intracellular replication. Cell Microbiol 4:341–355

Weber C, Kitayama J, Springer TA (1996) Differential regulation of beta 1 and beta 2 integrin avidity by chemoattractants in eosinophils. Proc Natl Acad Sci USA 93:10939–10944

Xia Y, Vetvicka V, Yan J et al (1999) The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. J Immunol 162: 2281–2290

Xu S, Huo J, Gunawan M et al (2009) Activated dectin-1 localizes to lipid raft microdomains for signaling and activation of phagocytosis and cytokine production in dendritic cells. J Biol Chem 284:22005–22011

Yago T, Shao B, Miner JJ et al (2010) E-selectin engages PSGL-1 and CD44 through a common signaling pathway to induce integrin alphaLbeta2-mediated slow leukocyte rolling. Blood 116: 485–494

Yamamura S, Handa K, Hakomori S (1997) A close association of GM3 with c-Src and Rho in GM3-enriched microdomains at the B16 melanoma cell surface membrane: a preliminary note. Biochem Biophys Res Commun 236:218–222

Ying M, Grimmer S, Iversen TG et al (2003) Cholesterol loading induces a block in the exit of VSVG from the TGN. Traffic 4:772–784

Yoshizaki F, Nakayama H, Iwahara C et al (2008) Role of glycosphingolipid-enriched microdomains in innate immunity: microdomain-dependent phagocytic cell functions. Biochim Biophys Acta 1780:383–392

Zarbock A, Lowell CA, Ley K (2007) Spleen tyrosine kinase Syk is necessary for E-selectin-induced alpha(L)beta(2) integrin-mediated rolling on intercellular adhesion molecule-1. Immunity 26:773–783

Zarbock A, Abram CL, Hundt M et al (2008) PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR gamma to induce slow leukocyte rolling. J Exp Med 205:2339–2347

Zimmerman JW, Lindermuth J, Fish PA et al (1998) A novel carbohydrate-glycosphingolipid interaction between a beta-(1–3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. J Biol Chem 273:22014–22020