Editorial

Membrane Rafts and Signaling

Signals triggered through the receptors on the plasma membrane of innate and adaptive immune cells are regulated in space and time (spatial-temporal). While the basic understanding of the temporal events of the signaling cascade initiated at the plasma membrane has remained a longstanding focus, the insights into the spatial distribution of signaling proteins and their re-organization during signaling process is under intense investigation. It has been proposed that membrane rafts on the plasma membrane provide a platform for the organization of signaling molecules during initiation and/or regulation of membrane signaling in a variety of cell types. While the merit of methods to investigate these nanometer size domains are being debated, the details of their role in signal transduction remain a hot topic of investigation. In a Keystone Symposium on "Lipid Rafts and Cell Function" in 2006 membrane rafts were defined as *small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions.* Small dynamic and compositionally heterogeneous nature of membrane rafts has been proposed to be central to their functional role. A number of signaling proteins are either housed in membrane rafts or traffic through these cholesterol-rich nano-domains during cell signaling. It has been suggested that small and dynamic nature of each membrane raft on an un-stimulated plasma membrane may be key to its existence as "incomplete signaling unit" and therefore contributing to the quiescent state of the cell. Coalescence of membrane rafts on the plasma membrane during cell stimulation allow congregation of signaling proteins in the rafts, thereby promoting their molecular interactions and generation of signals that cascade to the cell interior. Over the past decade it has become increasingly clear that signaling through pattern recognition receptors (PRR) in innate immune cells (e.g., dendritic cells (DC) and macrophages) and multi-chain antigen receptor in cells of adaptive immunity (e.g., B and T cells) either get initiated in membrane rafts or propagated through these nano-domains. While the details related to the involvement of membrane rafts are currently being worked-out, this new paradigm in cell signaling has direct implications in initiation/regulation of immune response during normal and abnormal immune responses. This thematic issue on "Membrane Rafts and Signaling" reviews some important aspects of raft biology and provides an additional possibility of rational drug design to interrupt or modulate signals in immune cells.

Kazuhisa Iwabuchi and colleagues from Juntendo University, Graduate School of Medicine in Chiba, focus their review on Lactosylceramide (LacCer, CDw17), an innate immune receptor expressed on neutrophils, that bind a variety of micro-organisms, including *Bordetella pertussis, Helicobacter pylori, and Candida albicans.* This review also provides insights into co-localization of LacCer and a Src kinase, lyn, in membrane rafts and its role in superoxide generation, chemotaxis, and non-opsonic phagocytosis. Katharina Gaus and her colleagues from University of New South Wales in Sydney discuss the role of membrane rafts in phagocytosis mediated through Fc receptors in macrophages, another component cell of innate immunity.

Helper $CD4^+$ T cells orchestrate adaptive immune response by providing requisite help to other immune cells. $CD4^+$ T cells are capable of rendering this help only after sensing a pathogen or allergen presented to them by antigen presenting cells. The two interacting cells at their contact site generate immunological synapse (IS). Immune receptors that drive T cell adhesion and activation show remarkable organization at the IS. Recent findings provide evidence for condensation of T cell membrane at the contact site with antigen presenting cells during these cellular interactions. Katharina Gaus and her colleagues highlight a connection between membrane rafts and process of plasma membrane condensation at the IS. The process of membrane condensation in T cells is dependent on polymerization of a cytoskeletal protein, actin. While inhibitors of cholesterol have been widely used to illustrate the importance of these membrane domains in variety of physiological processes, this review discusses the use of oxysterol 7-keto cholesterol (7-KC). This cholesterol molecule offers a perpendicular protruding ketone group in position 7 and its incorporation into the membrane prevents the formation of membrane rafts by decreasing the lipid order and increasing the bilayer polarity. 7-KC appears to be a promising agent for disrupting membrane proximal signaling events that either get generated in the membrane rafts or relayed through these nano-size domains.

A host of signalling proteins are recruited to plasma membrane after activation through the immune receptors. These membrane-bound signalling proteins allow the signals to cascade to the cell interior. Localization of phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to membrane rafts and its phosphorylation by phosphatidylinositol 3-kinase (PI 3-Kinase) is one critical step in this process. This additional phosphorylation of the inositol part of PIP_2 generates PIP_3 that specifically binds to and recruits signaling proteins with pleckstrin homology (PH) domain. Bill Rodgers and Corey Johnson from Oklahoma Medical Research Foundation, Oklahoma City, provide insights into how specific alterations in the PIP_2 pools in membrane rafts alter morphology of T cells, a process that is dependent on cytoskeletal protein, actin. They also review the information on critical role of PIP_2 pools present in the membrane in regulating cell motility, endocytosis, exocytosis, phagocytosis, and cell activation.

Biochemical characterization of membrane rafts requires their isolation from the plasma membrane. Traditionally non-ionic detergents have been used to isolate these nano-domains. Analysis of detergent resistant membrane rafts has suggested that the

T cell receptor (TCR)-αβ and its signalling components, in its phosphorylated forms, are localized to membrane rafts seconds after activation through the antigen receptor. Other proteins compartmentalized into these domains included cluster differentiation (CD)-3 ζ (p23) and zeta-associated protein (ZAP)-70 kinase that bind to and regulate a variety of signaling molecules critical for downstream signaling culminating into specific gene expressions including transcription of interleukin-2 (IL-2) gene, a key growth factor for T cells. Hai-Tao He and his colleagues from Université de la Méditerranée, Marseille, summarize findings from their group and discuss a recent approach they are using to identify and characterize the nano-size membrane domains enriched in TCRαβ and CD95, a cell death protein, on living cells using fluorescent correlation spectroscopy (FCS). This biophysical method allows assessment of membrane rafts with in the context of plasma membrane and overcomes the complications that arise by the use of non-ionic detergents.

Role of membrane rafts in signaling has also emerged by studying these membrane entities in tumor B cells. Daniel Hoessli and his colleagues from University of Geneva provide a comprehensive review on the role of membrane rafts in assembling signaling clusters with a src kinase, lyn, which is locked in an active state. In these neoplastic cells membrane rafts promote activation of NF-kB and formation of signalosomes inside the cell. Membrane rafts through these assembled signalosomes generate long lasting anti-apoptotic and pro-survival cellular conditions that favor malignancy. Agents disrupting the membrane raft dependence in generating survival signals could potentially be exploited therapeutically.

Agents that specifically target membrane rafts and disrupt congregation of signaling proteins in these nano-domains may also assist us to suppress allergic responses and autoimmune disorders. Thierry Chardes and his colleagues from Institut de Recherche en Cancérologie de Montpellier and Université Montpellier describe their findings on how epitope-directed anti-CD4 monoclonal antibody disrupts lipid-protein rheostat in membrane rafts by excluding some key signaling proteins during their assembly. They also extend the concept of raft-based therapeutics to other antibodies, sterol- and sphingolipid-modulating drugs, glycerophospholipid analogs, fatty acid modulators, and peptide-derived molecules. Their review highlights a novel mode of action of drugs through dietary or therapeutic interventions that target membrane rafts.

In summary, this issue provides a comprehensive overview of several lines of intense research being carried out in the field of "Membrane Rafts and Signaling". In the next several years we are likely to see an emergence of raft modulators with the potential to suppress abnormal innate and adaptive immune responses. New and improved biochemical and biophysical methods for study of membrane rafts will remain another focus of future research. Methods that allow throughput analysis of nano-domains coupled with their visualization in the context of plasma membrane remains an essential goal for deeper understanding of spatial-temporal regulation of membrane signaling. Hopefully this basic understanding will stimulate interest in targeting membrane rafts for rational design of agents capable of interrupting signaling that occur during abnormal immune responses. I would like to thank each contributor of this thematic issue for a wonderful contribution.

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