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Review Article

MEMBRANE CHOLESTEROL ORGANIZATION, REGULATION AND TRAFFICKING: CELLULAR CHOLESTEROL HOMEOSTASIS

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ABSTRACT: Cholesterol is an essential structural component of mammalian cell membranes and plays crucial roles in intracellular transport, cell signalling and regulation etc. Proper regulation of cholesterol homeostasis in the body is important for human health. Recently, plenty of new findings reveal the molecular mechanism of cholesterol uptake, which may provide new insights on our understanding of cholesterol homeostasis. In this review, we summarized recent progress in cholesterol biology and hoping to provide new perspectives on the regulation of cholesterol transport and metabolism.

ABBREVIATIONS: ER, endoplasmic reticulum; SCAP, sterol regulatory element binding protein cleavage activating protein; (HMG-CoAR), hydroxyl methyl glutaryl CoA (HMG-CoA) reductase; NPC1, Niemann–Pick C1 protein; SSD, sterol-sensing domain; SRE, sterol regulatory element; LDLR, low density lipoprotein receptor; APOE, Apoproteins E; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LXRs, liver X receptors; SR, scavenger receptors; SR-A, scavenger receptor-A; NADPH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; ACAT, acyl CoA: cholesterol acyltransferase; ACAT-1, Acyl coenzyme-A: cholesterol acyltransferase-1; FC, free cholesterol; M1, classical activation macrophage;M2, alternative activation macrophage; CEs, Cholesterol ester; (IFN)-γ, interferon; TLR, Toll-like receptor; Th2,T-helper, IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor (GM-CSF), LPS, lipopolysaccharide; TNF, tumor necrosis factor; (TGF)-β, transforming growth factor; HB-EGF, heparin binding epidermal growth factor; IGF, insulin-like growth factor; CC, chemokines; IDO, indole amine 2,3-dioxygenase.

INTRODUCTION

The lipid components of biological membranes are important for normal cell function, and their improper distribution or metabolism can have serious consequences for cells and organisms. Some of the important functions of membranes— such as providing a permeability barrier that separates compartments in eukaryotic cells — have been appreciated since the first observations of sub cellular organelles. Other functions, such as signalling by phosphoinositides, have also been studied for decades, but recent advances indicate new ways in which these signalling mechanisms can be regulated both spatially and temporally. In the past few years, several lines of evidence have shown that the biophysical properties of membrane bilayers have significant effects on the properties of membrane proteins (A G Lee, 2004). Changes in the organization of lipids can have profound effects on cellular functions such as signal transduction and membrane trafficking (Simons K *et al.,* 2004; Mukherjee S *et al.,* 2004; Holowka David *et al.,* 2005). Nevertheless, there is growing evidence that domains (i.e., localized regions with non-random lipid compositions) exist in biological membranes. Sorting of lipids appears to be need and demand of cholesterol sciences. Deeper understanding of cholesterol homeostasis leads to development therapeutic approaches to enhance better understanding of cholesterol and prevention of diseases. This review introduces general aspects of cholesterol biology in cell membranes and sources of cellular cholesterol along with signalling pathways regulation. Cholesterol transport among specific intracellular compartments and their communication with extracellular cholesterol donors and acceptors are discussed here, followed by remarks on cholesterol trafficking pathways in specialized way in macrophage cholesterol regulation and polarization.

CHOLESTEROL: Cellular Organization and Functions

Cholesterol plays a unique role among the many lipids in mammalian cells. Cholesterol is an essential constituent in mammalian cell membranes but the overall cholesterol: protein ratio varies considerably, depending on cell density (Takahashi Miwa *et al.,* 2007). Moreover, cholesterol is distributed heterogeneously between cellular membranes (Figure 1).

Cholesterol is one component of the plasma membrane that plays a critical role in the formation of membrane domains. In some cells, cholesterol is enriched in discrete areas of the plasma membrane (Gatfield John *et al.,* 2000; Millan Jaime *et al*., 2002; Pardo Mercedes *et al*., 2003; Vasanji A *et al*., 2004).
Cholesterol is enriched in the plasma membrane, where it typically accounts for 20–25% of the lipid molecules, with various phospholipids, sphingomyelin and glycolipids making up the remainder (Simons K *et al.*, 1988).
By contrast, the endoplasmic reticulum (ER) has low cholesterol content, with count as low as 1% of total cell cholesterol (Lange Y, 1991). The ER cholesterol concentration appears to control several functions that are related to the ER and ER–Golgi membrane transport, such as the operation of the sterol-homeostatic machinery (Goldstein JL *et al*., 2006), the activity of resident ER proteins and the exit of newly synthesized membrane proteins from the ER (Feng B *et al.* 2003; Ridsdale, A *et al.* 2006; Runz H *et al*., 2006). Intriguingly, sterol depletion in the ER inhibits the ER-to-Golgi transport of a secretory marker protein (Ridsdale A *et al.* 2006; Runz H *et al.,* 2006) but enhances the sterol regulator (sterol regulatory element binding protein cleavage activating protein: SCAP) (Nohturfft A *et al*., 1998). How this differential regulation is accomplished is not clear but it appears to involvement of sterol interference with COPII coat protein recruitment (Runz H *et al.,* 2006) (COPII are coated vesicle that are involved in transport from the endoplasmic reticulum to the Golgi).

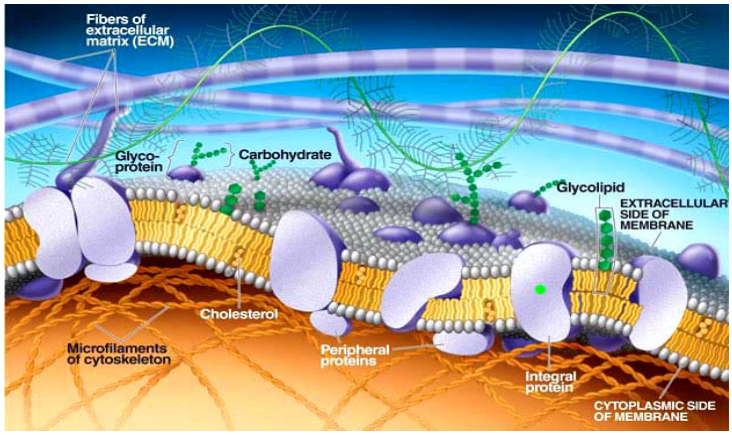

Figure 1: Fluid mosaic model of the plasma membrane

The functions of membrane cholesterol

Membranes must also be flexible enough to bend, for example, when budding to form vesicles or tubules, or fusing with other membranes during trafficking. Furthermore, many biological membranes have a lateral in homogeneity (micro domains) that can be used to bring signalling molecules (both lipids and membrane proteins) together or to keep them apart under various conditions (Simons K *et al.,* 2004; Mukherjee S *et al.,* 2004; Holowka David *et al.,* 2005).
The membranes provide a permeability barrier to allow different ion and solute concentrations to exist on each side of the membrane. This permits specialized functions in various organelles and maintains trans membrane electrical potentials. The membranes of mammalian cells serve several functional roles that are carried out simultaneously.
Cholesterol affects cellular processes by interacting with other membrane lipids as well as with specific proteins. Because of the rigid sterol backbone, cholesterol is preferentially positioned in close proximity to saturated hydrocarbon chains of neighbouring lipids, as these are more inflexible and elongated compared with those of unsaturated lipids (Simons K *et al.,* 2004).This allows different ion and solute compositions to exist on each side of the membrane.
Some membrane proteins bind tightly to cholesterol (Murata M *et al.* 1995). Moreover, several key proteins that is involved in cellular cholesterol homeostasis or trafficking (such as hydroxyl methyl glutaryl CoA (HMG-CoA) reductase (HMG-CoAR), SCAP and Niemann–Pick C1 protein (NPC1) harbour a conserved five-transmembrane helix domain called the sterol-sensing domain (SSD) (Nohturfft A *et al.,* 1998). However, the precise function of this domain is unclear. Several proteins belonging to these families have an affinity for cholesterol but many of them also bind other sterols as well as other lipids, and display complex phenotypes in lipid homeostasis. Thus, it is often difficult to determine whether they function as direct sterol transporters or as sterol sensors that orchestrate cellular functions, such as lipid homeostasis, vesicular trafficking and signalling (see below). For instance, sterol-induced alterations in membrane biophysical properties could indirectly affect protein functions (A G Lee, 2004).

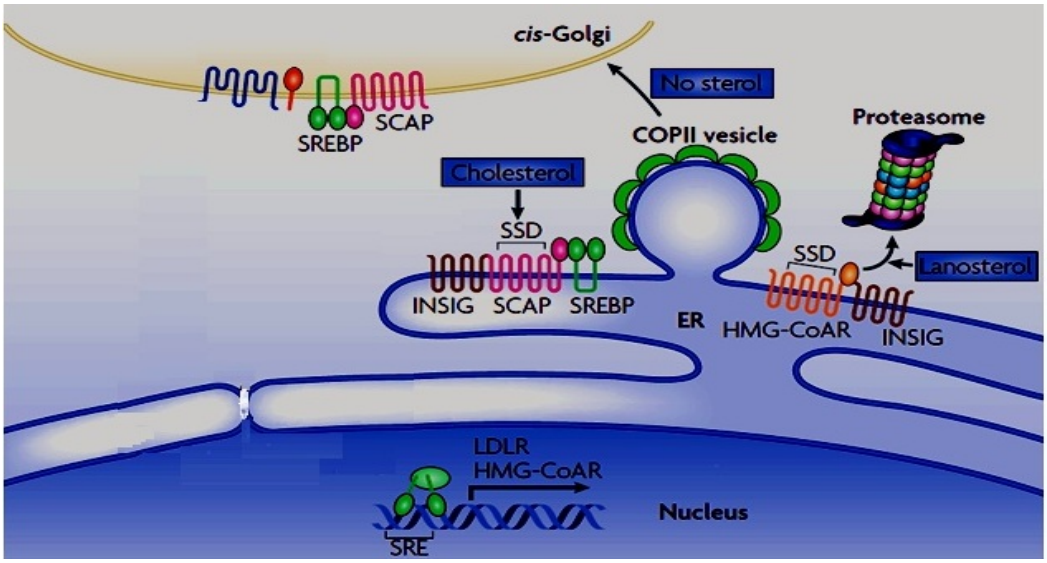

Figure 2: Cholesterol metabolism regulation by sterol regulatory element binding protein (SREBP). Under presence of sterol in the endoplasmic reticulum (ER), the ER retention protein INSIG prevents entry of the SREBP–SCAP (SREBP cleavage activating protein) complex to COPII-coated vesicles. Transport of SREBP to the Golgi is essential for proteolytic release of the transcription factor, which is then transported to the nucleus to activate sterol-regulated genes (such as hydroxyl methyl glutaryl CoA reductase (HMG-CoAR) and the low density lipoprotein receptor (LDLR). HMG-CoAR is also post-transcriptionally regulated by sterol, with INSIG binding of the protein leading to its proteasomal degradation.

Sources of Cellular Cholesterol

The *de novo* cholesterol synthesis versus dietary intake for total body cholesterol has been estimated as a ratio of ~70:30 (Grundy SM 1983). All nucleated cells can synthesize cholesterol from acetyl Co-A through the mevalonate pathway. The first sterol intermediate in this pathway is lanosterol, which is further trimmed by several enzymes to form cholesterol (Figure 3). In some cells, the post-lanosterol steps operate slowly, resulting in the accumulation of cholesterol precursors and/or their secretion from cells (Lange Y, 1991; Lusa S *et al*., 2003). The physiological relevance of this phenomenon is poorly understood. The rate-limiting step of the mevalonate pathway is the conversion of HMG-CoA to mevalonate by HMG-CoAR. Both this and several other enzymes that function in later steps of cholesterol synthesis are integral ER membrane proteins. The ER also harbours enzymes for some of the key cholesterol processing steps, Oxysterols are typically present in cells in minor amounts (roughly 1:1000 compared to cholesterol) where as steryl esters may accumulate to levels exceeding that of unesterified cholesterol in some cells, such as macrophages (Maxfield Frederick R *et al*., 2005) . Additional hydroxyl groups render a sterol more hydrophilic.

Cholesterol metabolism

All carbon atoms of cholesterol derive from the simple building block acetate — as described by (Konrad Bloch *et al.,* 1964). The rate-limiting enzyme of the pathway is hydroxyl methyl glutaryl CoA reductase (HMG-CoAR; the target of statins), which catalyses the synthesis of mevalonate and is under tight regulation (Figure 3). Cholesterol can be fatty acylated to form cholesteryl esters in all cells (cholesteryl oleate), or it can be oxidized to form oxysterols by enzymatic reactions or by auto-oxidation in all cells (25-hydroxycholesterol) or oxidized to bile acids in hepatocytes or oxidized to steroid hormones in steroidogenic cells (pregnenolone).

Dietary cholesterol and transfer between tissues.

Cholesterol obtained from food sources is transported from the intestine to the liver from which it is transported throughout the body. Dietary cholesterol is absorbed form enterocytes of the small intestine, along with triglycerides, to chylomicrons. Some triglycerides are hydrolysed in the circulation and new apoproteins, such as **APOE,** are added to produce chylomicrons which are taken up by hepatocytes. Hepatocytes, in roll secrete lipids which are very low density lipoprotein **(VLDL)** particles that are processed in the circulation into low density lipoprotein (**LDL**), the chief lipoprotein that delivers cholesterol to the peripheral cells. If there is extra cholesterol in hepatic tissue, it can be released in form of high density lipoprotein (HDL). HDL returns the lipid to the liver by a process called reverse cholesterol transport. Liver secreted the cholesterol as the bile (either as in form of cholesterol or after it has been metabolized in form of bile acids) that enters the small intestine. Cholesterol and bile salts are either reabsorbed in enter hepatic cycle or excreted into faeces.

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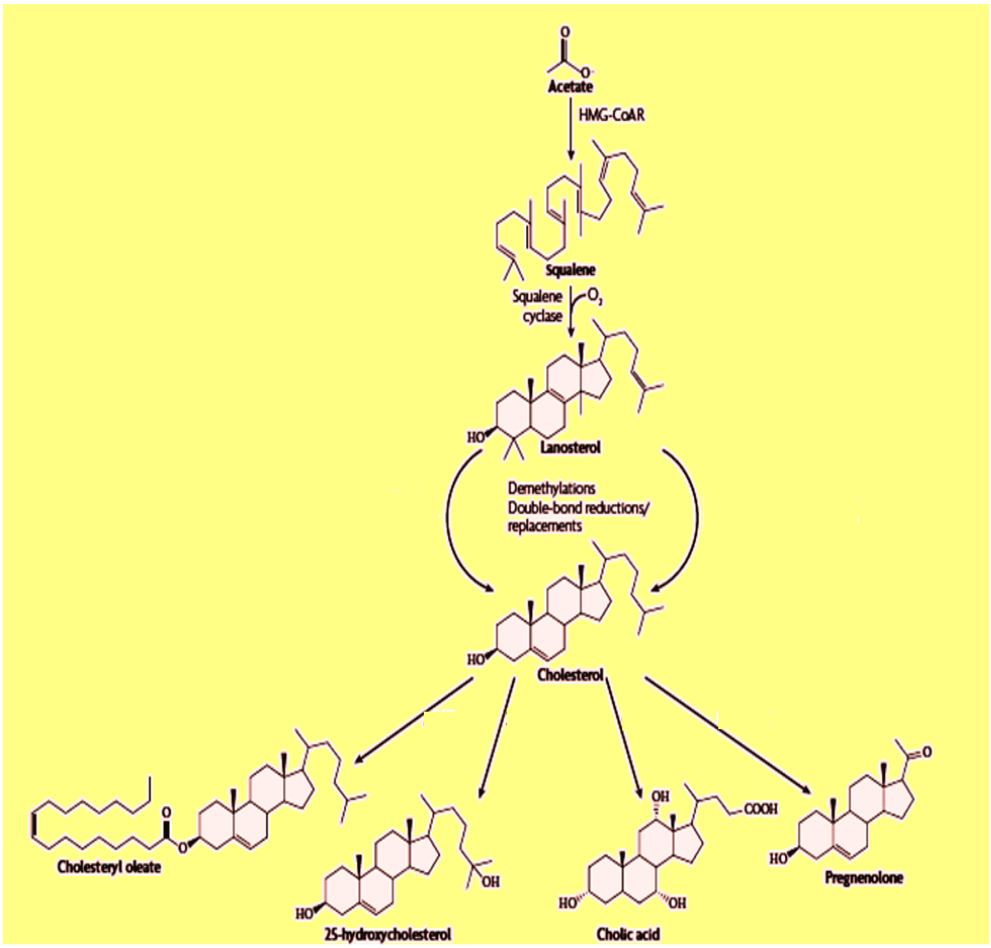

Fig. no 3: *De novo* cholesterol synthesis

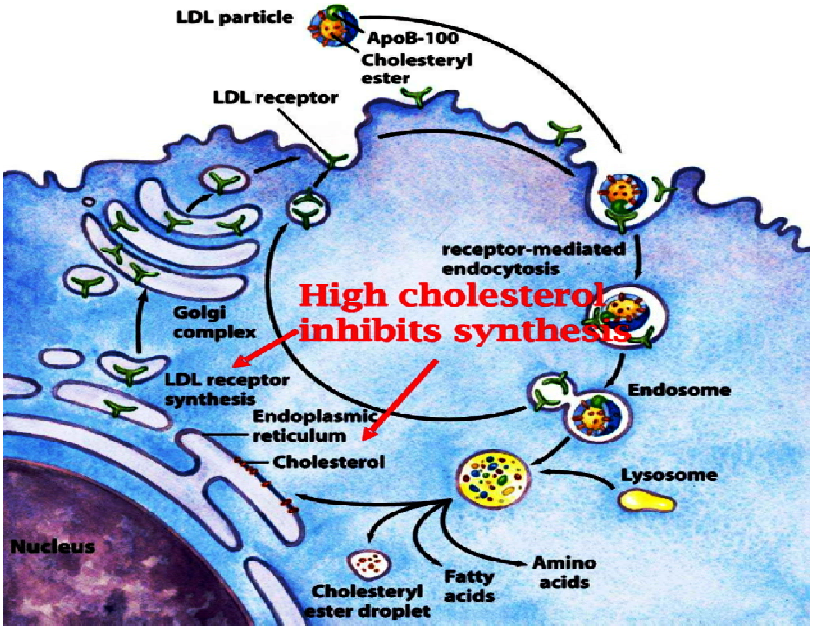

Figure 4: Transcriptional regulation of cholesterol homeostasis by LDL receptor.

Transcriptional Control of Cholesterol Levels

Cellular cholesterol synthesis, are regulated in a complex manner by two main nuclear receptor systems, sterol regulatory element binding proteins (SREBPs) and liver X receptors (LXRs). SREBP activation increases the transcription of gene products that function to increase cellular cholesterol levels (Goldstein JL *et al.,* 2006).

The transcriptional regulation of cholesterol homeostasis by SREBP is one of the elucidated mechanisms in cholesterol cell biology. SREBP resides in the ER and is activated in state of sterol-poor conditions bind to Golgi complex, undergoes proteolytic processing (Goldstein JL *et al.,* 2006) (Figure 2). The processed fragment is then transported into the nucleus, where it switches on the transcription of HMG-CoAR and other sterol-regulated genes such as the LDL receptor (LDLR). SCAP is a SREBP Golgi escort protein and INSIG is an ER anchor protein. Binding of cholesterol to SCAP and binding of 25-hydroxycholesterol to INSIG causes these chaperones to bind one another (Radhakrishnan A *et al.,* 2004; Sun LP *et al.,* 2007) and impart signal in SCAP inaccessible to the COPII complex so that SCAP no longer enters ER–Golgi transport vesicles (Sun LP *et al.,* 2005). An additional stage of regulation is communicated by the cholesterol precursor lanosterol, which directs HMG-CoAR to proteasomal degradation (Song BL *et al.,* 2005).

The LXR activation facilitates reverse cholesterol transport; that is a processes leading to cholesterol removal from the peripheral cells and increases biliary sterol secretion (Tontonoz P *et al.,* 2003). LXR is activated by oxysterols, but how the crosstalk between membrane cholesterol levels, oxysterol generation and LXR activation orchestrated is not well understood. Interestingly, recent data indicate that a specific ER-resident ORP may modulate this (Yan Daoguang *et al.,* 2008).

How Does Cholesterol Induce Cellular Changes?

Cholesterol loading does not increase actin-based ruffling in suspended cells, when the ruffling induced by cholesterol loading of attached cells is blocked by incubation with fucoidan, a ligand for scavenger receptors as noted in study of (Maxfield Frederick R *et al.,* 2006) These data indicate that cholesterol itself does not serve as a signalling molecule to induce the effects observed in this study (Maxfield Frederick R *et al.,* 2006). Instead, increasing membrane cholesterol levels may alter plasma membrane organization in a way that potentiates signalling by adhesion molecules. Maxfield in suggested that scavenger receptors (SR) contribute to the cholesterol-sensitive signals that lead to changes in cell function. It is interesting to note that scavenger receptors can activate signalling pathways leading to actin polymerization and focal adhesion formation, presumably by binding ligands in the extracellular matrix (Post S R *et al.,* 2002). The Rho family GTP ases, Rho, cdc 42, and Rac, are known to be major mediators of signalling leading to actin reorganization (Etienne-Manneville S *et al.,* 2002; Hall A *et al.,* 1998). The Rho GTPases are thought to regulate the formation of distinct actin filament-containing structures. The morphological responses in macrophages induced by membrane cholesterol loading resembled the effects of Rac activation; investigated work evidenced the participation of Rac signalling in cholesterol induced membrane ruffling (Maxfield Frederick R *et al.,* 2006).

RHO Family Proteins and the Actin Cytoskeleton

Small GTPases of the Rho family are pivotal regulators of signalling networks that are activated by chemokine and cytokine receptors as well as other receptor types (Weiss-Haljiti C *et al.,* 2004). Who wish to see more detail, many excellent reviews exist and here we are trying to reporting some example. The Rho family is part of the Ras super family of small (around 21 kDa) GTP-binding proteins. Data suggested, 15 mammalian members of the Rho family have been identified: Rho (A, B, C, D, E, G), Rac (1, 2, 3), Cdc42 (two alternatively spliced variants of the same gene with different carboxy-terminal sequences), Rnd1/Rnd6, Rnd2/Rho7, TC10, and TTF. Of the mammalian proteins, the best regarded as for regulation of actin organization are RhoA, Rac1, and Cdc42. Rho was the first member of this family to be cloned in 1985, followed a few years later by Rac and Cdc42. The most frequently used tool for studying Rho function is C3 transferase, an exoenzyme from *Clostridium botulinum*, which ADP ribosylates and inactivates Rho. Treatment of many cell types with C3 transferase induces loss of stress fibers, and this was the first indication that Rho influences the actin cytoskeleton. Subsequently, Rac was also shown to regulate actin organization, and at the same time was independently purified as an essential cofactor for the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) in phagocytic cells (Grundy SM, 1983).

The roles of Rho, Rac, and Cdc42 in regulating actin organization were first characterized in detail in Swiss 3T3 fibroblasts. These cells have proven to be a good model system for analyzing rapid changes occur in actin cytoskeleton, because when confluent and serum-starved they lose practically all of the two most prominent actin filament-containing structures found in fibroblasts: stress fibers and lamellipodia. Stress fibers are bundles of actin filaments associated with myosin-II filaments and other proteins, forming contractile fibers. They terminate at the plasma membrane in focal adhesions, where transmembrane integrins are clustered and associate both with extracellular matrix proteins outside the cell and with a large number of proteins inside the cell (Lange Y *et al.,* 1991). Cdc42 can induce Rac-mediated lamellipodium formation, and Rac can induce Rho-mediated stress fiber formation (Yamauchi Yoshio *et al.,* 2007).

RHO Regulation in Macrophage Migration during Cholesterol Homeostasis (Loading)

Macrophages take up cholesterol from the matrix-bound LDL aggregates and become lipid filled foam cells (Osterud B *et al.,* 2003). Oxidatively modified LDL strongly inhibited the chemotactic responses of mouse resident peritoneal macrophages, (Quinn MT *et al.,* 1985; Quinn MT *et al.,* 1987) and oxidized or aggregated LDL changed the organization of the F-actin cytoskeleton and decreased the ability of macrophages to generate locomotors forces (Zerbinatti C V *et al.,* 2003) However, the molecular mechanism(s) by which lipoprotein interactions caused these changes in macrophages is not understood. The interactions of macrophages with aggregated and matrix-bound LDL have some distinctive features that are not apparent when macrophages bind soluble LDL. When macrophages begin to engulf aggregated matrix-bound LDL particles, they maintain prolonged contact with the LDL aggregates (Buton X *et al.,* (1991). During this prolonged contact there can be rapid transfer of cholesterol from the LDL aggregates to the macrophage. As a result, transient increases in the cholesterol content of macrophage plasma membranes may result. These findings suggest two possible ways that lipoprotein interactions could lead to changes in macrophage F-actin organization and migration: (1) The binding of modified lipoproteins to their receptors could initiate signals that negatively regulate the cytoskeletal changes required for migration; or (2) Cholesterol transfer from lipoproteins could modulate macrophage plasma membrane cholesterol levels, and this change in membrane cholesterol could alter signal transduction mechanisms in the macrophage.

Membrane Cholesterol and Cell Migration in Macrophage

Despite having potentiating effects on membrane activity and F-actin reorganization, cholesterol loading caused the inhibition of macrophage migration. Cell migration is a highly integrated, multi-step process consisting of cell polarization, membrane extension at the front of the cell, regulated formation and release of adhesions along the length of the cell, and retraction of the cell rear (uropod). These steps are orchestrated in part by the interactive regulation of the Rho-family GTPases (Weiss-Haljiti C *et al.,* 2004; Nobes CD *et al.,* 1999; Raftopoulou M *et al.,* 2004). Data clearly shows that, Rac activity and localization are altered after cholesterol loading, and it is likely that the activities of the other Rho family members are also affected by changes in membrane cholesterol levels.

Several studies have shown that plasma membrane organization is critical for cell migration (Vasanji A, *et al.,* 2004; Manes S *et al.,* 1991; Pierini LM *et al.,* 2003) and previous works on neutrophils showed that depleting membrane cholesterol inhibited neutrophil migration because the cells were unable to form membrane extensions and polarize (Pierini LM *et al.,* 2003). Further studies are required for understanding the exact defect in the migration of cholesterol-loaded macrophages.

The observations reported here indicate that increased membrane cholesterol causes dramatic effects on the actin cytoskeleton in macrophages associating with extracellular matrix. It is possible that after initial contact of a macrophage with lipoproteins in the vessel wall, cholesterol transfer to the cell causes changes similar to those observed in above lines. The increased membrane ruffling and extensions could increase the contact of the macrophage with the lipoprotein deposit, leading to further cholesterol delivery.

The decreased motility of the cell would note in the region of the lipoprotein deposit. Additional studies will be required to determine to what extent contact with lipoprotein particles causes local increases in membrane cholesterol levels and the consequences of these local changes for cell function.

Cholesterol Homeostatic Mechanisms

Organisms must maintain the proper functioning of their membranes in response to various changes. In humans, one of the most noted factors affecting the membrane architecture is dietary intake of cholesterol and fats, which are delivered to cells throughout the body through lipoproteins (Goldstein JL *et al.,* 2001). Because cholesterol is a key regulator of lipid organization, its cellular concentration must be maintained within a narrow range, and cells have a variety of mechanisms for accomplishing this. Transport of sterol through vesicular and non vesicular pathways reflected in changes in cholesterol levels in many organelles. A rapid response to increasing cholesterol levels is the esterification of excess cholesterol by an ER enzyme, acyl CoA: cholesterol acyltransferase (ACAT) (Chang TY *et al.,* 1997). The esterified cholesterol is stored well in cytoplasmic lipid droplets. The cholesterol esters in the droplets are hydrolysed by neutral cholesterol ester hydrolases, which in some cells by hormone-sensitive lipase that also hydrolyses triglycerides in fat cells (Yeaman SJ *et al.,* 2004). The cholesterol released from the droplets can be utilising for cell membranes and, in steroidogenic cells, for steroid hormone synthesis. The activity of ACAT is regulated by cholesterol levels (Tabas Ira, 2002). This regulation occurs at two levels: ACAT is allosterically regulated by cholesterol (Zhang Y *et al.,* 2003), and cholesterol loading of cells promotes more rapid efflux of sterol from the plasma membrane (Wustner Daniel *et al.,* 2005) which could increase the rate of delivery to ACAT in the ER (Lange Y et al., 2004). As reported earlier, many genes involved in cholesterol metabolism are regulated by SREBP (Brown MS *et al.,* 1999). This releases the cytosolic domain of SREBP, which is then translocated into the nucleus and regulates the transcription of many genes, including the LDL receptor and HMG-CoA reductase as these are consider the rate-limiting enzyme in cholesterol synthesis. Thus, this coordination regulates both the synthesis of cholesterol and its uptake through lipoproteins. A third level of cholesterol regulation within cells is provided by cholesterol efflux mechanisms (Figure 2).

In the past few years, the molecular mechanisms for cellular export of cholesterol have begun to be understood, but there are still significant gaps in our knowledge concerning how these processes operate and how they are regulated. The key extracellular acceptors for cholesterol are high-density lipoproteins (HDLs) and one of their associated Apo-lipoproteins, Apo A-I (Tall AR *et al.,* 2002)

This regulation can help to maintain the proper biophysical properties of the cell membranes, but it is uncertain whether membrane bilayer properties themselves directly regulate these biosynthetic pathways.

Macrophage Cholesterol Homeostasis and Macrophage Polarization

Cholesterol homeostasis in macrophages and other peripheral cells is maintained by a balance between the influx and efflux pathways. Cholesterol influx occurs through receptor and non-receptor-mediated-uptake of both normal and modified lipoproteins (Figure 4). While the uptake of LDLs via the LDL receptor is limited due to feedback inhibition of LDL receptor expression by cellular cholesterol levels, modified LDL uptake by scavenger receptors, namely scavenger receptor-A (SR-A) and CD36, is largely unregulated and contributes substantially to foam cell formation.

In addition, nonreceptor-mediated uptake pathways such as phagocytosis of aggregated LDLs and macropino cytosis of native (Kruth Howard S, 2002; Kruth Howard S *et al.,* 2002; Kruth Howard S *et al.,* 2005) or modified LDLs (Choi Soo-Ho *et al.,* 2009) can also potentially contribute to lipid accumulation in macrophage foam cells. Cholesterol ester (CEs) associated with the lipoproteins are hydrolyzed in late endosomes lysosomes to release free cholesterol (FC), which then traffics to and integrates into the plasma membrane (Tabas Ira, 2000). Excess membrane cholesterol and a fraction of LDL-derived FC is transported to the endoplasmic reticulum where it is re-esterified by Acyl coenzyme-A: cholesterol acyltransferase-1 (ACAT1) and stored in cytoplasmic lipid droplets. Under conditions of unregulated or increased uptake of modified LDL it leads to an excessive accumulation of CE present as cytoplasmic lipid droplets, giving the cells their prominent characteristic 'foamy' appearance and look.

Concept of Regulation of Macrophage Polarization and Function

Macrophages are innate immune cells and their attendance in most tissues. Under physiological conditions, macrophages cooperates tissue homeostasis by producing trophic factors, clearing cell debris, and preventing inflammatory in response to various environmental stresses (Murray PJ *et al.,* 2011).

Infection or tissue injury activates macrophage host defense functions that include microbial killing and production of cytokines and chemokines. Macrophage polarization states are defined by the inducing stimulus and by the ensuing patterns of gene expression, which determine function (Gordon S *et al.,* 2010; Lawrence T *et al.,* (2011; Mosser DM *et al.,* 2008; Sica A *et al.,* 2012). Activated macrophages polarize towards various functional phenotypes depending on the pathogen and cytokines expressed in the microenvironment (Gordon S *et al.,* 2010; Lawrence T *et al.,* 2011; Mosser DM *et al.,* 2008; Sica A *et al.,* 2012). The best characterized macrophage activation phenotypes are classical activation (also termed M1) induced by interferon (IFN)-γ and microbial products such as Toll-like receptor (TLR) ligands, and alternative activation (M2) induced by the T-helper (Th2) cytokines interleukin (IL)-4 and IL-13. M1 macrophages are effective at host defense and clearing pathogens, and M2 macrophages are important for resolution of inflammation and tissue repair. The classical M1 and M2 activation phenotypes represent two ends of a functional spectrum of macrophage polarization states that are induced by multiple factors and are characterized by expression of transcriptional modules that underlie specialized functions (Gordon S *et al.,* 2010; Lawrence T *et al.,* 2011; Mosser DM *et al.,* 2008; Sica A *et al.,* 2012).

In vivo, macrophage phenotype is heterogeneous, and multiple polarization states have been described; it is useful to conceptualize these states as existing on a spectrum of overlapping phenotypes and gene expression patterns related to the original classification of M1 and M2 (Mosser DM *et al.,* 2008; Sica A, *et al.,* 2012; Murray PJ *et al.,* 2011). Thus, various M1-like macrophages [induced by IFNs, granulocyte macrophage colony-stimulating factor (GM-CSF), lipopolysaccharide (LPS), and other microbial products] are effective at killing microbes and producing inflammatory cytokines, but have the potential to cause toxicity and collateral tissue damage. Core aspects of the M1-like group of phenotypes are high expression of key M1 effector molecules, such as the cytokines tumor necrosis factor (TNF), IL-1, and IL-12, antimicrobial molecules, reactive oxygen and reactive nitrogen intermediates, and IFN-induced genes such as theTh1-recruiting chemokines CXCL9 and CXCL10 (Mosser DM et al., 2008). By contrast, M2-related macrophages [induced by IL-4/13, IL-10, transforming growth factor (TGF)-β, glucocorticoids, and immune complexes] promote tissue function under physiological conditions, preserve function during times of stress, restrain and resolve inflammation after infection or injury, and promote repair and wound healing. Core genes expressed by M2 macrophages include scavenger receptors, growth factors [heparin binding epidermal growth factor (HB-EGF) and insulin-like growth factor (IGF)], Th2 chemokines (CCL18 and CCL22), and suppressors of inflammation and immunity such as IL-10 and indole amine 2,3-dioxygenase (IDO) (Gordon S *et al.,* 2010). Polarization of macrophages in response to various signals functions are summarized (Gordon S *et al.,* 2010; Lawrence T et al., (2011; Mosser DM *et al.,* 2008; Sica A *et al.,* 2012; Murray PJ *et al.,* 2011).

CONCLUSIONS AND FUTURE DIRECTIONS

In the past several years there has been increased interest in the role of the cholesterol in biological membranes. The role of cholesterol and sterol derivatives as signalling molecules is well established, but important new discoveries on the signalling roles of these molecules continue to be made. In this review, we have focused on the more subtle role of lipids and cholesterol in regulating the biophysical properties of membranes and how this affects cellular cholesterol homeostasis.

Significant advances in the multifarious area of cellular cholesterol trafficking and compartmentalization have been reached substantial advancement in past few years. New regulators of these processes have been identified and are being studied in increasingly nowadays. Modern sophisticated developed methods for high-resolution imaging of lipid movement and for *in vivo* tissue imaging provide resources for higher study. These improvements will continue to make the study of cholesterol transport and distribution much more accessible to research.

Several issues await further studies. Parameters for cholesterol movement and interaction with other lipids in the membranes of living cells need to be characterized. These should essentially help to interpret and make predictions about larger-scale sterol movement between organelles or exchange between membranes and lipoproteins. For most sterol transport processes, only a limited number of proteins that are involved have been identified. There is still little understanding of the interplay between the different proteins, and considering the paucity of characterized protein–protein interactions, important players must be missing. Further studies on members of large lipid-binding protein families, should reveal new components. New readouts for sterol transport and distribution that are compatible with high-throughput screens should also be developed.

In addition, there is an apparent need to understand the orchestration of sterol balance in complex systems. Besides increasing our insights into the physiology of cholesterol trafficking, the information obtained should help to develop improved strategies for controlling cholesterol-related pathologies. In conclusion, it is clear that macrophages are extremely versatile cells that can perform diverse functions due to their ability to adapt to a wide range of tissue micro-environmental signals by displaying equally diverse functional phenotypes. Besides the evidence for such polarized response in vitro, study of macrophages in vivo in several pathologies like inflammation, atherosclerosis, diabetes, hyperlipidemia and metabolic disease, indeed, demonstrates the macrophage polarization of cells as an important driving force in pathogenesis. Thus, identifying the molecular determinants of macrophage polarization will not only be important to our understanding of the basis of a wide range of diseases but also open new options for their therapeutic targeting. Much work needs to be done to better characterize the biophysical properties of biological membranes and to study the effects that these properties have on membrane. Finally, recent review will opened a new door of various diseases, which hold promise for inducing long-term disease remission.

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Conflict of interest

The authors have no conflicts of interest to declare.

REFERENCES

Bloch Konrad (1964). The biological synthesis of cholesterol. Nobel Lecture, December 11: 78-100.

Buton X. (1991). Unique cellular events occurring during the initial interaction of macrophages with matrix-retained or methylated aggregated low density lipoprotein (LDL): Prolonged cell surface contact during which ldl-cholesteryl ester hydrolysis exceeds ldl protein degradation. J Biol Chem 274: 32112–32121.

Brown MS, Goldstein JL (1999). A proteolytic pathway that controls the cholesterol content of membranes, cells and blood. Proc Natl Acad Sci USA 96: 11041–11048.

Choi Soo-Ho., (2009). Lipoprotein accumulation in macrophages via toll-like receptor-4-dependent fluid phase uptake. Circ Res 104: 1355–1363.

Chang TY, Chang CC, Cheng D (1997). Acyl-coenzyme A: cholesterol acyltransferase. Annu Rev Biochem 66: 613–638.

Etienne-Manneville S, Hall A (2002). Rho GTPases in cell biology. Nature 420: 629–635.

Feng B. (2003). The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nature Cell Biol **5**: 781–792.

Grundy SM (1983). Absorption and metabolism of dietary cholesterol. Annu Rev Nutr 3: 71–96.

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

Goldstein JL, Brown MS (2001). Molecular medicine: The cholesterol quartet. Science 292: 1310–1312.
Goldstein JL, DeBose-Boyd RA, Brown MS (2006). Protein sensors for membrane sterols. Cell 124: 35–46.
Gordon S, Martinez FO (2010). Alternative activation of macrophages: mechanism and functions. Immunity 32: 593–604
Hall A (1998). Rho GTPases and the actin cytoskeleton. Science 279: 509–514.
Holowka David et al. (2005). Lipid segregation and IgE receptor signalling: A decade of progress. Biochim Biophys Acta 1746: 252 – 259.
Kruth Howard S (2002). Sequestration of aggregated low-density lipoproteins by macrophages. Curr Opin Lipidol 13(5):483–488.
Kruth Howard S, Huang Wei, I Ishii, Zhang Wei-Yang (2002). Macrophage foam cell formation with native low density lipoprotein. J Biol Chem 277(37): 34573–34580.
Kruth Howard S., (2005). Macropinocytosis is the endocytic pathway that mediates macrophage foam cell formation with native low density lipoprotein. J Biol Chem 1280(3): 2352–2360.
Lange Y (1991). Disposition of intracellular cholesterol in human fibroblasts. J Lipid Res 32: 329–339.
Lusa S, Heino S, Ikonen E (2003). Differential mobilization of newly synthesized cholesterol and biosynthetic sterol precursors from cells. J Biol Chem 278:19844–19851.
Lee AG (2004). How lipids affect the activities of integral membrane proteins. Biochim Biophys Acta 1666: 62–87.
Lange Y, Ye J, Steck TL (2004). How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. Proc Natl Acad Sci USA 101:11664–11667.
Lawrence T, Natoli G. (2011). Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 11: 750–761.
Manes S. (1991). Membrane raft microdomains mediate front-rear polarity in migrating cells. E M B O J 18: 6211–6220.
Murata, M. (1995). VIP21/caveolin is a cholesterol-binding protein. Proc Natl Acad Sci USA 92: 10339–10343.
Millan Jaime, Montoya Maria C, Sancho David, Sanchez-Madrid Francisco, Alonso Miguel A (2002). Lipid rafts mediate biosynthetic transport to the T lymphocyte uropod sub domain and are necessary for uropod integrity and function. Blood 99: 978-984.
Mukherjee S, Maxfield FR (2004). Membrane domains. Annu Rev Cell Dev Biol 20: 839–866.
Maxfield Frederick R,Tabas Ira (2005). Role of cholesterol and lipid organization in disease. Nature 438: 612-621.
Maxfield Frederick R, Qin Chunbo, Nagao Tomokazu, Grosheva Inna, Pierini Lynda M (2006). Elevated Plasma Membrane Cholesterol Content Alters Macrophage Signaling and function. Arterioscler Thromb Vasc Biol 26: 372-378.
Mosser DM, Edwards JP (2008). Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8: 958–969.
Murray PJ, Wynn TA. (2011). Obstacles and opportunities for understanding macrophage polarization. J Leukoc Biol 89 : 557–563.
Murray PJ, Wynn TA (2011). Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11 (2011) 723–737.
Nohturfft A, Brown MS, Goldstein JL (1998). Sterols regulate processing of carbohydrate chains of wild-type SREBP cleavage-activating protein (SCAP), but not sterol-resistant mutants Y298C or D443N. Proc Natl Acad Sci USA 95: 12848–12853.
Nobes CD, Hall A (1999). Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol 144: 1235–1244.
Osterud B, Bjorklid E (2003). Role of monocytes in atherogenesis. Physiol Rev 83: 1069 –1112.
Post S. R, Gass C, Rice S, Nikolic D, Crump H, Post GR (2002). Class-A scavenger receptors mediate cell adhesion via activation of G (i/o) and formation of focal adhesion complexes, J Lipid Res 43: 1829 –1836.
Pardo Mercedes, Nurse Paul, (2003). Eqsuatorial retention of the contractile actin ring by microtubules during cytokinesis. Science 300: 1569.
Pierini LM, Eddy RJ, Fuortes M, Seveau S, Casulo C, Maxfield FR (2003). Membrane lipid organization is critical for human neutrophil polarization. J Biol Chem 278: 10831–10841.
Quinn MT, Parthasarathy S, Steinberg D (1985). Endothelial cell-derived chemotactic activity for mouse peritoneal macrophages and the effects of modified forms of low density lipoprotein. Proc Natl Acad Sci USA 82: 5949 –5953.
Quinn MT, Parthasarathy S, Fong LG, Steinberg D (1987). Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci U S A 84 : 2995–2998.
Raftopoulou M, Hall A (2004). Cell migration: Rho GTPases lead the way. Dev Biol 265: 23–32.

Radhakrishnan A, Sun LP, Kwon HJ, Brown MS, Goldstein JL (2004). Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. Mol Cell 15: 259–268.

Ridsdale, A. (2006). Cholesterol is required for efficient endoplasmic reticulum-to-Golgi transport of secretory membrane proteins. Mol Biol Cell 17: 1593–1605.

Runz H, Miura K, Weiss R, Pepperkok P (2006) Sterols regulate ER-export dynamics of secretory cargo protein ts-O45-G, EMBO. J. 25 : 2953–2965.

Simons K, Van Meer G (1988). Lipid sorting in epithelial cells. Biochemistry 27: 6197-6202.

Simons K, Vaz WL (2004). Model systems, lipid rafts, and cell membranes. Annu Rev Biophys Biomol Struct 33: 269–295.

Sun LP, Li L, Goldstein JL, Brown MS (2005). Insig required for sterol-mediated inhibition of Scap/SREBP binding to COPII proteins in vitro. J Biol Chem 280: 26483–26490.

Song BL, Javitt NB, DeBose-Boyd RA (2005). Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. Cell Metab 1: 179–189.

Sun LP, Seemann J, Goldstein JL, Brown MS (2007). Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: Insig renders sorting signal in Scap inaccessible to COPII proteins. Proc Natl Acad Sci USA 104: 6519–6526.

Sica A, Mantovani A (2012). Macrophage plasticity and polarization: in vivo veritas. J Clin Invest 122 (2012) 787–795.

Tabas Ira (2000). Cholesterol and phospholipid metabolism in macrophages. Biochem Biophys Acta 1529(1–3): 164–174.

Tall AR, Costet P, Wang N (2002). Regulation and mechanisms of macrophage cholesterol Efflux. J Clin Invest 110 899–904.

Tabas Ira (2002). Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. J Clin Invest 110: 905–911.

Tontonoz P, Mangelsdorf DJ (2003). Liver X receptor signaling pathways in cardiovascular disease. Mol Endocrinol 17: 985–993.

Takahashi Miwa, Murate Motohide, Fukuda Mitsunori, Sato Satoshi B, Ohta Akinori, Kobayashi Toshihide (2007). Cholesterol controls lipid endocytosis through Rab11. Mol Biol Cell 18: 2667–2677.

Vasanji A, Ghosh PK, Graham LM, Eppell SJ, Fox PL (2004). Polarization of plasma membrane micro viscosity during endothelial cell migration. Dev Cell 6: 29–41.

Weiss-Haljiti C et al., (2004). Involvement of phosphoinositide 3-kinase gamma, Rac, and PAK signalling in chemokine-induced macrophage migration. J Biol Chem 279 : 43273– 43284.

Wustner Daniel, Mondal Mousumi, Tabas Ira, Maxfield Frederick R (2005). Direct Observation of Rapid internalization and intracellular transport of sterol by macrophage foam cell, Traffic 6 (2005) 396–412.

Yeaman SJ (2004). Hormone-sensitive lipase—new roles for an old enzyme. Biochem J 379: 11–22.

Yamauchi Yoshio, (2007). Plasma membrane rafts complete cholesterol synthesis by participating in retrograde movement of precursor sterols, J Biol Chem 282: 34994–35004.

Yan Daoguang. (2008). OSBP-related protein 8 (ORP8) suppresses ABCA1 expression and cholesterol efflux from macrophages. J Biol Chem 283: 332–340.

Zerbinatti C V, Gore RW (2003). Uptake of modified low-density lipoproteins alters actin distribution and locomotor forces in macrophages. Am J Physiol Cell Physiol 284: C555–C561.

Zhang Yi, Yu Chunjiang, Liu Jay, Spencer Thomas A., Chang Catherine C Y, Chang Ta-Yuan (2003) Cholesterol is superior to 7-ketocholesterol or 7 alph+a-hydroxycholesterol as an allosteric activator for acyl-coenzyme A:cholesterol acyltransferase 1. J Biol Chem 278:11642–11647.