Modulation of Chemokine Receptor Function by Cholesterol: New Prospects for Pharmacological Intervention

Daniel F. Legler, Christoph Matti, Julia M. Laufer, Barbara D. Jakobs, Vladimír Purvanov, Edith Uetz-von Allmen, and Marcus Thelen

Biotechnology Institute Thurgau at the University of Konstanz, Kreuzlingen, Switzerland (D.F.L., C.M., J.M.L., B.D.J., V.P., E.U.A.); Konstanz Research School Chemical Biology, University of Konstanz, Germany (D.F.L., C.M., J.M.L.); and Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland (M.T.)

Received October 7, 2016; accepted January 9, 2017

ABSTRACT

Chemokine receptors are seven transmembrane-domain receptors belonging to class A of G-protein-coupled receptors (GPCRs). The receptors together with their chemokine ligands constitute the chemokine system, which is essential for directing cell migration and plays a crucial role in a variety of physiologic and pathologic processes. Given the importance of orchestrating cell migration, it is vital that chemokine receptor signaling is tightly regulated to ensure appropriate responses. Recent studies highlight a key role for cholesterol in modulating chemokine receptor activities. The steroid influences the spatial organization of GPCRs within the membrane bilayer, and consequently can tune chemokine receptor signaling. The effects of cholesterol on the organization and function of chemokine receptors and GPCRs in general include direct and indirect effects (Fig. 1). Here, we review how cholesterol and some key metabolites modulate functions of the chemokine system in multiple ways. We emphasize the role of cholesterol in chemokine receptor oligomerization, thereby promoting the formation of a signaling hub enabling integration of distinct signaling pathways at the receptor-membrane interface. Moreover, we discuss the role of cholesterol in stabilizing particular receptor conformations and its consequence for chemokine binding. Finally, we highlight how cholesterol accumulation, its deprivation, or cholesterol metabolites contribute to modulating cell orchestration during inflammation, induction of an adaptive immune response, as well as to dampening an anti-tumor immune response.

Introduction

Chemokine receptors and their ligands, the chemokines, are key orchestrators of cell migration. They control numerous physiologic processes, including organogenesis, homeostatic leukocyte trafficking, and host immune responses to pathogens. Moreover, chemokine receptors also contribute to pathologic processes, such as metastasis formation. In addition, chemokine receptors can act as coreceptors for human immunodeficiency virus entry. Chemokine receptors belong to class A of the G-protein-coupled receptor (GPCR) superfamily (Bachelier et al., 2014), which constitute the largest group of cell surface receptors. They consist of seven transmembrane-spanning α-helical structures with the N-terminus, together with three loops being exposed to the extracellular environment and the C-terminus, which in concert with the intracellular loops is responsible for transmitting signals. GPCRs are encoded by more than 800 genes in the human genome (Fredriksson et al., 2003) and represent the most successful target family of pharmacological drugs (Cooke et al., 2015). GPCRs signal through heterotrimeric GTPases consisting of a Gα-subunit, a Gβ-subunit, and a Gγ-subunit, whereby the Gα-subunit brings most of the specificity to downstream effectors. The human genome codes for 31 Gα-, eight Gβ-, and 14 Gγ-subunits of the G-protein. Upon ligand binding, the GPCR acts as a nucleotide exchange factor, displacing GDP off the Gα-subunit of the heterotrimeric G-protein complex and enabling the loading with GTP. Subsequently, the GTP-loaded Gα-subunit dissociates from the Gβγ-heterodimer, both of which can trigger downstream signaling complexes. From a signaling point of view, chemokine receptors can be divided into two groups: the classic or conventional chemokine receptors that couple to heterotrimeric G-proteins for downstream signaling controlling
cell migration, and the atypical or decoy chemokine receptors that are scavenging chemokines to form chemotactic gradients in a G-protein-independent manner and that do not transmit signals involved in cell migration. Classic chemokine receptors can be loosely classified into two functional groups, inflammatory and mainly homeostatic chemokine receptors, based on whether they mediate altered leukocyte recruitment to sites of injury or inflammation, or whether they promote mostly homeostatic leukocyte trafficking and organ development, but can, under inflammatory conditions, also contribute to host defense (Mazzucchelli et al., 1999; Bachelerie et al., 2014; Zgraggen et al., 2014). According to the chemokine ligands that classic chemokine receptors bind, they are referred to as CC chemokine receptors (CCRs) (CCR1-10), CXC chemokine receptors (CXCRs) (CXCR1-6 and CXCR8), CX3CR1, and XCR1, whereas atypical chemokine receptors are termed ACKR1–4 (Bachelerie et al., 2014). Since molecular mechanisms of signal transduction events elicited by atypical chemokine receptors are far from being understood, this review article focuses primarily on the interplay of classic chemokine receptors with membrane lipids.

**Turning on Classic Chemokine Receptors**

Chemokine binding to its cognate receptor occurs in at least two steps in which the ligand initially interacts with the N-terminus and the three extracellular loops of the receptor followed by the insertion of the N-terminus of the chemokine into the minor pocket of the receptor (Thiele and Rosenkilde, 2014). In general, ligand binding to GPCRs leads to the rearrangement of the transmembrane helices, resulting in conformational changes in the cytoplasmic domains. For signal transduction, the changes stabilize an active receptor conformation required for G-protein coupling (Tesmer, 2016). As proposed for many GPCRs, the constitutive association of the G-protein alpha subunit to the receptor is required for signal transduction. As originally shown for CXCL18-mediated neutrophil activation (Thelen et al., 1988), most signaling events downstream from chemokine receptors can be inhibited by treatment of cells with Bordetella pertussis toxin, which through ADP ribosylation prevents binding of the heterotrimeric G-protein to activated G-protein-coupled receptors (Ogilvie et al., 2004). Along this line, pretreatment of naive T cells with B. pertussis toxin also completely blocks their ability to arrest on high endothelial venules and home to lymph nodes (Warnock et al., 1998). In contrast, pretreatment of effector T cells with B. pertussis toxin does not abrogate cell arrest on inflamed skin vessels, providing evidence that effector T cells can bypass chemokine-mediated Gαi-signaling (Shulman et al., 2012). Although classic chemokine receptors have been shown to couple to G-proteins other than Gαi in vitro cell systems, the biologic relevance of alternative G-protein coupling remains largely unclear. Noteworthy, dendritic cells (DCs), but not naive T cells, exploit a CD38/Gq-dependent signaling pathway in addition to Gαi for CC chemokine receptor 7 (CXCR7) and CXC chemokine receptor 4-dependent cell migration (Shi et al., 2007).

Besides coupling to heterotrimeric G-proteins, activated chemokine receptors like most GPCRs recruit GPCR kinases (GRKs) that phosphorylate multiple serine and threonine residues mainly located at the C-terminus of the receptor (Vroon et al., 2004; Balabanian et al., 2008; Busillo et al., 2010; Barker and Benovic, 2011; Gurevich et al., 2012; Raghunanshi et al., 2012; Tarrant et al., 2013). Subsequently, β-arrestins bind with high affinity to serine/threonine-phosphorylated GPCRs, resulting in quenching of heterotrimeric G-protein signaling and targeting of the receptor for clathrin-mediated endocytosis, which instigates unique

**Effects of cholesterol on membrane and chemokine receptors.** Cholesterol is integrated into phospholipid membranes, thereby altering their composition from a heterogeneous fluid membrane with high mobility to a more rigid and stiff membrane with lipid and protein patches. Cholesterol may alter the receptor mobility directly by interacting with its transmembrane domains as shown for the β-adrenergic receptor (Cherezov et al., 2007) and indirectly by altering the membrane composition and turning it more rigid, thereby reducing the interactions of the receptor with other membrane proteins. Swiss-Model (Bordoli et al., 2009; Biasini et al., 2014) and PyMol were used for modeling CCR7 (based on 2LNL) (Park et al., 2012). Phospholipid structures were retrieved from the LIPID MAPS Structure Database (Sud et al., 2007).
signaling cascades involving both MAP kinases and Src kinases (Vroon et al., 2004; Lefkowitz and Shenoy, 2005). A recent study provides evidence that a class B GPCR can form an intracellular supercomplex composed of a single GPCR, β-arrestin, and G-protein. Hence, a single class B GPCR is able to simultaneously interact with a heterotrimeric G-protein and with β-arrestin, resulting in sustained signaling from an internalized receptor (Thomsen et al., 2016). Whether chemokine receptors can also form such supercomplexes and whether intracellular signaling contributes to cell locomotion remains to be determined. In the case of CCR7 it was shown that GRK3 and GRK6 are recruited to the activated receptor. Of note, CCL19-mediated activation of CCR7 leads to a robust phosphorylation of the receptor by both GRK3 and GRK6, whereas CCR7 phosphorylation by its second ligand, CCL21, is much weaker and solely mediated by GRK6 (Zidar et al., 2009). By contrast, both ligands are able to similarly recruit β-arrestin to CCR7 and to stimulate extracellular signal–regulated kinase 1/2 activation (Otero et al., 2008; Zidar et al., 2009). However, interestingly, only CCL19 promotes efficient CCR7 internalization (Otero et al., 2006), which is in agreement with the notion that GRK3 is required for GPCR internalization (Reiter et al., 2012). CCL19 dissociates from the internalized receptors and is sorted for lysosomal degradation. CCR7 instead recycles back to the plasma membrane to reparticipate in chemokine sensing and cell migration (Otero et al., 2006). Therefore, it has been proposed that receptor trafficking contributes to signaling involved in cell guidance. In contrast, CCL21 hardly induces CCR7 internalization but facilitates integrin activation, cell adhesion, haptotokinesis, and diapedesis (Schumann et al., 2010; Hauser and Legler, 2016).

Chemokine Receptor Oligomerization as a Hub to Integrate Distinct Signaling Pathways

Spatial organization of chemokine receptors into dimers and higher-ordered oligomers further adds to the complexity of possible GPCR arrangements, and consequently modulation of signaling (Thelen et al., 2010; Stephens and Handel, 2013). CCR2 was the first chemokine receptor shown to form functional dimers (Rodríguez-Frade et al., 1999). Dimers of chemokine receptors are presumably formed during biosynthesis since they exist constitutively and in the absence of ligands (Issafras et al., 2002) and are detectable in small vesicles during transport from the endoplasmic reticulum to the Golgi (Singer et al., 2001). Chemokine receptors can form homo- as well as heteromers. Noteworthy, the organization of chemokine receptors in higher-order oligomers has been shown (Sohy et al., 2009) and the arrangement of dimers within oligomeric structures with and without direct physical interaction has been discussed (Thelen et al., 2010). Both allosteric inhibition as well as cooperative and synergistic activation of such chemokine receptor dimers and oligomers have been reported for various receptor pairs (Thelen et al., 2010; Stephens and Handel, 2013). Only recently, molecular details have been identified on how dimerization and oligomerization can modulate chemokine receptor signaling for the chemokine receptor CCR7 (Hauser et al., 2016). A combination
of biochemical cysteine crosslinking, molecular modeling, and directed evolutionary screening revealed that a hydrophobic interaction surface proximate to the NPXXY motif within transmembrane domain 7 of CCR7 is critical for receptor dimerization and oligomerization. Reducing the hydrophobic interaction surface by site-directed mutagenesis of single amino acids led to the identification of CCR7 mutants with impaired dimerization capacities. In contrast, enlarging the hydrophobic interaction surface near the NPXXY motif revealed CCR7 variants with superoligomerizing properties. Interestingly, one of the identified CCR7 superoligimerizer is, in fact, a naturally occurring CCR7 single-nucleotide polymorphism. Strikingly, T cell lines expressing CCR7 superoligimerizers displayed higher migratory activities toward CCL19 and CCL21 compared with cell lines expressing wild-type receptors, despite similar chemokine binding and G-protein-activation properties. Concomitantly, cells expressing oligomerization-defective mutants migrate even less. The enhanced migration efficiency of oligomeric CCR7 could be attributed to a chemokine-mediated Src kinase activity. More precisely, Src was shown to constitutively interact with CCR7 oligomers, which was significantly reduced in oligomerization-defective mutants. Ligand binding to the chemokine receptor oligomer led to Src-dependent phosphorylation of the tyrosine residue within the highly conserved DRY motif located at the transition between transmembrane domain 3 and the second intracellular loop of the receptor. Tyrosine-phosphorylated CCR7 in turn acted as a docking site for further downstream signaling molecules harboring Src homology 2 (SH2) domains, such as the tyrosine-phosphatase SHP2. Noteworthy, mutating the tyrosine residue within the DRY motif or inhibiting Src kinases by PP2 diminished cell migration. This study established that CCR7 dimers and other higher-order oligomers form a platform enabling integration of G-protein- and Src-dependent signaling pathways in parallel, resulting in more effective cell migration (Hauser et al., 2016). Whether this signaling integration is specific for CCR7 or is of general nature remains to be investigated. However, it has been reported that the tyrosine residue within the DRY motif of CCR2 becomes phosphorylated by Janus kinase 2 following receptor stimulation with CCL2 (Mellado et al., 1998), while the kinetics of Janus kinase 2–mediated CCR2 phosphorylation suggests that the kinase is activated downstream from G-protein stimulation (Thelen and Baggioleti, 2001). The involvement of Janus kinase activity in general chemokine receptor-mediated signaling and cell migration has been reported to be controversial (Moriguchi et al., 2005).

**Association of GPCRs with Membrane Cholesterol**

Chemokine receptors, as any other GPCR, are integral membrane proteins. The interaction of membrane lipids with the seven-transmembrane domain-spanning receptors represents an important determinant in their structure and function. As a major cell membrane lipid, cholesterol plays a crucial role in membrane organization and its dynamics, sorting, and hence function (Simons and Ikonen, 2000; Ikonen, 2008). One cholesterol molecule can span roughly half of a lipid bilayer that preferentially interacts with saturated hydrocarbon chains of sphingolipids and phospholipids. The unique puckered four-ring structure of cholesterol confers special biophysical properties that increase cohesion and packing of neighboring lipids and proteins, and hence cholesterol is thought to function as a dynamic glue. Many studies that address the relationship between cholesterol and a GPCR rely on experiments with cholesterol depleting agents, such as methyl-β-cyclodextrin (MβCD), and cholesterol synthesis inhibitors, such as members of the statin family. However, these methods do not allow discriminating between whether cholesterol directly interacts with the GPCR or not and if it may indirectly interferes with GPCR signaling by affecting other pathways. The effects of cholesterol on GPCR organization and function include direct and indirect effects (Chini and Parenti, 2009; Paila and Chattopadhyay, 2009). Direct effects are those that arise from cholesterol physically interacting with the GPCR, whereas indirect effects are caused by alterations in the physicochemical properties of the membrane that embed the receptor (Fig. 1). The latter includes thickness and rigidity of the lipid bilayer. Thus, changes in cholesterol levels affect the lateral mobility of GPCRs within the lipid bilayer, as well as in signaling molecules that are membrane associated through lipidation, such as the Ga and the Gβγ-subunits of the G-protein and the Src kinases. Both effects have in common that they participate in modulating the GPCR’s conformation and dynamics (Oates and Watts, 2011; Sengupta and Chattopadhyay, 2015). The importance of cholesterol for GPCRs is supported by the fact that addition of cholesterol is mandatory to increase the stability of numerous GPCRs upon solubilization, purification, and crystallization (Ghosh et al., 2015). Evidence for physical interaction between cholesterol and GPCRs derives from the crystal structure of the β2-adrenergic receptor (β2AR), where two cholesterol molecules associated with a receptor monomer (Hanson et al., 2008). Moreover, crystals derived from β2AR bound to a partial inverse agonist revealed a symmetric arrangement of dimeric receptors (Cherezov et al., 2007). Remarkably, more than two-thirds of the β2AR-specific symmetry interface is mediated by ordered lipids consisting of six cholesterol and two palmitic acid molecules per receptor dimer. The notion that both cholesterol and the GPCR are synthesized in the endoplasmic reticulum could explain why GPCR dimerization and its stabilization by cholesterol might act as quality control for receptor export from the endoplasmic reticulum (Terrillon and Bouvier, 2004). Based on β2AR structures, a consensus cholesterol binding motif was postulated that was found in almost every second class A GPCR (Hanson et al., 2008) but not in chemokine receptors. Subsequent molecular dynamics simulation of GPCR-cholesterol interactions revealed several sites on certain GPCRs with high cholesterol occupancy that is dynamic, rather than the presence of static cholesterol binding sites (Sengupta and Chattopadhyay, 2015). Confusingly, a number of GPCRs can functionally—in terms of ligand binding and G-protein-activation abilities—be expressed in cholesterol-free membranes of Escherichia coli providing evidence that the effect of cholesterol on GPCR organization and function appears to be receptor dependent (Oates and Watts, 2011; Sengupta and Chattopadhyay, 2015).
Role of Cholesterol in Modulating Chemokine Receptor Functions

As mentioned previously, chemokine receptors do not possess a consensus cholesterol binding motif (Hanson et al., 2008). This is supported by the solved structures of the two chemokine receptors, CXCR4 (Wu et al., 2010) and CCR5 (Qin et al., 2015), where no specific cholesterol binding sites have been identified. Nonetheless, both purified chemokine receptor complexes were reconstituted into a lipidic cubic phase that contained cholesterol for crystallization. Evidence that cholesterol plays a critical role in chemokine receptor functions derives from experiments with cholesterol-modulating drugs. Cholesterol depletion from membranes reversibly attenuated chemokine binding and abrogated chemokine receptor signaling, as shown consistently for CCR5 (Nguyen and Taub, 2002; Signoret et al., 2005). This is in line with findings that inclusion of cholesterol increased chemokine binding to solubilized receptors, as exemplified for the CXCL12-CXCR4 pair (Babcock et al., 2003; Palmesino et al., 2016). Regulation of chemokine receptors by cholesterol came into the spotlight with the discovery that CCR5 and CXCR4 act as coreceptors for human immunodeficiency virus infection and that cellular cholesterol is critically involved in initiating the fusion of the virus envelope with the host cell membrane (Simons and Ehehalt, 2002). In fact, the viral glycoprotein gp120 was found to copatch with the chemokine receptor CXCR4 or CCR5, together with CD4 in cholesterol-rich domains of the host cell (Mañas et al., 2000; Ono and Freed, 2001). Thereby, viral binding seems to promote clustering of cell surface receptors as well as of cholesterol-rich membrane patches. The cholesterol-dependent lateral assembly of such a protein complex is key to initiating the fusion of the virus envelope with the host cell membrane (Ono and Freed, 2001).

The concept of cholesterol acting as dynamic glue enabling efficient chemokine receptor signaling has recently been supported experimentally. Moderately reducing cellular cholesterol levels using low amounts of MβCD, cholesterol oxidase, or statins substantially increased the presence of CCR7 oligomers on the surface of DCs and T cells (Hauser et al., 2016). Of note, cholesterol depletion from membranes reversibly attenuated chemokine binding and abrogated chemokine receptor signaling, as shown consistently for CCR5 (Nguyen and Taub, 2002) and CCR7. This was due to impaired T cell trafficking; namely, by impaired lymph node homing and consequently lack of T cell priming, as well as hampered lymph node egress and migration to sites of inflammation (Luchtetal. et al., 2010). In addition, CCR7 induction on fat-laden macrophages, the foam cells, facilitated their emigration to lymph nodes, resulting in regression of atherosclerosis in ApoE-deficient mice (Trogan et al., 2006). Consequentially, preclinical studies using statins to inhibit cholesterol synthesis in ApoE-deficient mice revealed regression of atherosclerosis via activation of a CCR7-dependent emigration of foam cells from plaques (Feig et al., 2011). Furthermore, statins not only profoundly inhibited secretion of inflammatory chemokines (CCL2, CCL3, and CCL4) by tumor necrosis factor–stimulated human vascular endothelial cells, but also downregulated the expression of the corresponding chemokine receptors on human macrophages (Veillard et al., 2006). In addition, statins are able to prevent isoprenylation of the Rho family of small G-proteins (Cordle et al., 2005), resulting in the functional inactivation of these molecules known to regulate cell polarization and migration. Hence, the results from these experimental studies may help to understand why beneficial effects of statins in clinical trials to treat atherosclerosis patients were found to go beyond lowering lipid and cholesterol levels (Charo and Taub, 2011). It is worth mentioning that membrane cholesterol levels can alter the activity of many membrane proteins, not only subendothelial layer of the arterial wall in combination with an inflammatory immune response contributes to progressive narrowing and hardening of the arteries leading to atherosclerosis. Of note, accumulation of cholesterol in atherosclerotic plaques may give rise to the formation of cholesterol crystals. Macrophages exposed to cholesterol crystals at inflamed arteries locally produce the inflammatory chemokines CCL2, CCL3, and CCL5, which recruit additional monocytes/macrophages, DCs, and T cells, which contribute to chronic inflammation and disease progression in a CCR2-dominated manner (Boring et al., 1998). Noteworthy, cholesterol crystals also induce complement-dependent inflammasome activation, resulting in secretion of further inflammatory chemokines (Samstad et al., 2014). In animal models for atherosclerosis, namely, in apolipoprotein E (ApoE)-deficient mice or low-density lipoprotein receptor-deficient mice, local skin resident DCs not only promote dermal inflammation but also display systematic altered migration behavior (Angeli et al., 2004). Paradoxically, although inflammatory signals are known to induce CCR7 expression on DCs required for lymph node homing (Ohl et al., 2004), DC migration from skin to lymph nodes in ApoE-deficient mice was severely impaired (Angeli et al., 2004). Interestingly, impaired DC migration was attributed to inhibitory signals generated by a platelet-activating factor or oxidized low-density lipoprotein cholesterol serving as mimetic of the platelet-activating factor. Noteworthy, triggering of liver X receptors (LXRs), which are oxysterol-activated transcription factors, on mouse or human DCs resulted in significant down regulation of CCR7 expression and hence impaired DC migration (Villablanca et al., 2010; Bruckner et al., 2012), which might contribute to the refraining DCs in the vicinity of atherosclerotic plaques. However, the role of CCR7 in atherosclerosis is more complex. Reduced atherosclerotic plaques were observed in mice lacking both low-density lipoprotein receptor and CCR7. This was due to impaired T cell trafficking; namely, by impaired lymph node homing and consequently lack of T cell priming, as well as hampered lymph node egress and migration to sites of inflammation (Luchtetal. et al., 2010). In addition, CCR7 induction on fat-laden macrophages, the foam cells, facilitated their emigration to lymph nodes, resulting in regression of atherosclerosis in ApoE-deficient mice (Trogan et al., 2006). 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Several lines of evidence indicate that CX3CR1, which is expressed on platelets and inflammatory monocytes/macrophages, together with its membrane-bound ligand CX3CCL1 (fractalkine) present in plaques are involved in high-density lipoprotein cholesterol-induced proinflammatory signaling in atherosclerotic lesions (Flierl and Schäfer, 2012). Tumors evade the immune system mainly through mechanisms conditioning their microenvironment and cholesterol metabolites were shown to play an important role in dampening anti-tumor immune responses. In line with this finding, tumor cells secrete LXR ligands, presumably oxysterols (Villalblanca et al., 2010). Tumor cell–derived LXR ligands impaired CCR7 expression of tumor-resident DCs, thereby preventing their emigration from the tumor and homing to draining lymph nodes. As a consequence, tumor-derived antigens are not transported to lymph nodes and hence not presented to tumor-specific T cells. Moreover, tumor-derived cholesterol metabolites trigger LXRs on DCs, leading to inhibited transcription and expression of CCR7, and thereby severely impairing induction of a specific anti-tumor immune response (Villalblanca et al., 2010). Noteworthy, tumor progression is often associated with inflammation. The inflammatory mediator and arachidonic metabolite prostaglandin E2 (PGE2) significantly contributes to enhanced and efficient DC migration toward CCR7 ligands (Kabashima et al., 2003; Legler et al., 2006). In this context, PGE2 was found to downregulate LXR expression on DCs, rendering them less sensitive to oxysterols (Bruckner et al., 2012). In fact, CCR7-driven DC migration is conversely regulated upon cell encountering by PGE2 and oxysterol. Shifting the balance between PGE2 and oxysterols presumably determines whether the immune system tolerates the tumor or initiates an adaptive immune response against the tumor.

Successful elimination of pathogenic intruders relies on highly coordinated processes involving both innate and adaptive immune responses. Acute inflammation is the host’s major and intimate reaction to invading pathogens. Locally residing DCs sense the intruder and transport antigens to the draining lymph nodes to launch a pathogen-specific immune response. As introduced previously, DC and T cell homing to lymph nodes is controlled by CCR7 and its ligands (Fürster et al., 2008; Hauser and Legler, 2016). Interestingly, PGE2 is one of the very first proinflammatory mediators found at sites of infection (Legler et al., 2010). DCs encountering a pathogen take up antigens derived from the pathogen and induce expression of CCR7. This enables DCs to migrate along a preexisting CCL21 gradient toward the next lymphatic vessel (Weber et al., 2013). Exposure of DCs to inflammatory signals, and even more pronounced in the presence of PGE2, enhances the presence of CCR7 oligomers on the cell surface, which correlates with enhanced cell migration capacities (Hauser et al., 2016). Interestingly, human monocyte-derived DCs exposed to inflammatory signals and PGE2 were found to downregulate major genes coding for enzymes of the cholesterol biosynthesis, metabolism, and transport pathways, suggesting that inflammation-mediated lowering of cellular cholesterol levels facilitates CCR7 oligomerization and efficient DC migration. This notion is supported by findings that moderately lowering cholesterol levels by treating cells with statins, MβCD, or cholesterol oxidase promoted oligomerization of CCR7. Furthermore, treating T cells with moderate concentrations of MβCD transiently enhanced T cell migration toward CCR7 ligands (Hauser et al., 2016). However, reducing cholesterol levels further affects both membrane organization as well as the conforma- tional integrity of the chemokine receptor (Nguyen and Taub, 2002). Strikingly, also positioning of lymphocytes in lymph nodes can be controlled by cholesterol derivatives. Unexpectedly, the orphan G-protein-coupled receptor EB12 was identified as a specific receptor for oxysterols (Hannedouche et al., 2011; Liu et al., 2011). The receptor was identified as an Epstein-Barr virus-induced gene, together with EB11 (Birkenbach et al., 1993), which was renamed to CCR7 after the identification of its ligand CCL19 (Yoshida et al., 1997; Legler et al., 2014). Oxysterols recruit EB12-expressing B cells and guide them to the T/B boundary in follicular areas of the spleen (Liu et al., 2011). Hence, oxysterols control B cell positioning for mounting a T cell-dependent antibody-mediated immune response (Pereira et al., 2009; Gatto et al., 2013; Jarrossay and Thelen, 2013). Interestingly, an in vitro study additionally suggests that EB12 heterodimerizes with CXCXR5, the key receptor mediating lymphocyte recruitment to B cell follicles in secondary lymphoid organs (Barroso et al., 2012).

In summary, balanced cholesterol levels within the plasma membrane is decisive for proper chemokine receptor function. Moderate fine tuning of cholesterol levels holds the promise to open new ways to potentially interfere with chemokine receptor-mediated cell migration in a number of pharmacological relevant situations, e.g., in promoting guided cell recruitment to fight invading pathogens or cancer, or in blocking cell locomotion to prevent autoimmunity or metas-tasis formation.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Legler, Matti, Laufar, Jakobs, Purvanov, Uetz-von Allmen, Thelen.

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