HOST CELL RESISTANCE

Cholesterol in quarantine

Host cell cholesterol is often exploited by pathogens for entry and egress. Two new studies elucidate a new interferon-inducible mechanism by which cells limit plasma membrane cholesterol to promote antibacterial defense.

Eric V. Dang, Hiten D. Madhani and Russell E. Vance

Cholesterol is a key structural lipid in mammalian cell membranes that influences lipid bilayer packing and fluidity. Unlike other lipids, cholesterol cannot be catabolized to drive ATP production. Thus, molecular pathways that carefully monitor and control cellular cholesterol abundance are needed in order to prevent its overaccumulation. As a unique feature of animal membranes, cholesterol has been targeted by pathogens for their pathogenesis. Two recent reports — by Zhou et al.[1], in this issue of *Nature Immunology*, and Abrams et al.[2], in *Nature Microbiology* — uncover a new mechanism whereby the cholesterol metabolite 25-hydroxycholesterol (25-HC) provides protection against bacterial infection and secreted bacterial toxins by depleting plasma membrane (PM) cholesterol.

Cholesterol biosynthesis and uptake are controlled by SREBP (sterol regulatory element–binding protein) transcription factors[3]. SREBPs are endoplasmic reticulum (ER)-resident transmembrane proteins that partner with the escort protein SCAP. When ER cholesterol levels drop below 5 mole percent of the total lipid, the SCAP–SREBP complex translocates to the Golgi apparatus where proteases release the cytosolic transcription factor domain of SREBP, allowing it to travel to the nucleus and drive gene expression. Conversely, when ER cholesterol levels rise above 5 mole percent of total lipid, the SCAP–SREBP complex binds to the anchor protein Insig, anchoring the complex in the ER. This ER-dependent mechanism is, at first glance, counterintuitive, as 40–90% of the total cellular cholesterol resides in the PM, whereas the ER only contains ~1% of the total cholesterol. However, it is now appreciated that PM cholesterol is not homogenously distributed but is instead divided into distinct pools, including a 'free', accessible pool and a sequestered pool[4]. The size of the sequestered pool appears to be relatively constant, whereas the size of the freely accessible pool fluctuates according to whether the cell is in cholesterol replete or depleted conditions. Crucially, excess cholesterol is transported from the accessible PM pool to the ER, providing a mechanism by which PM cholesterol can be monitored by ER-resident proteins.

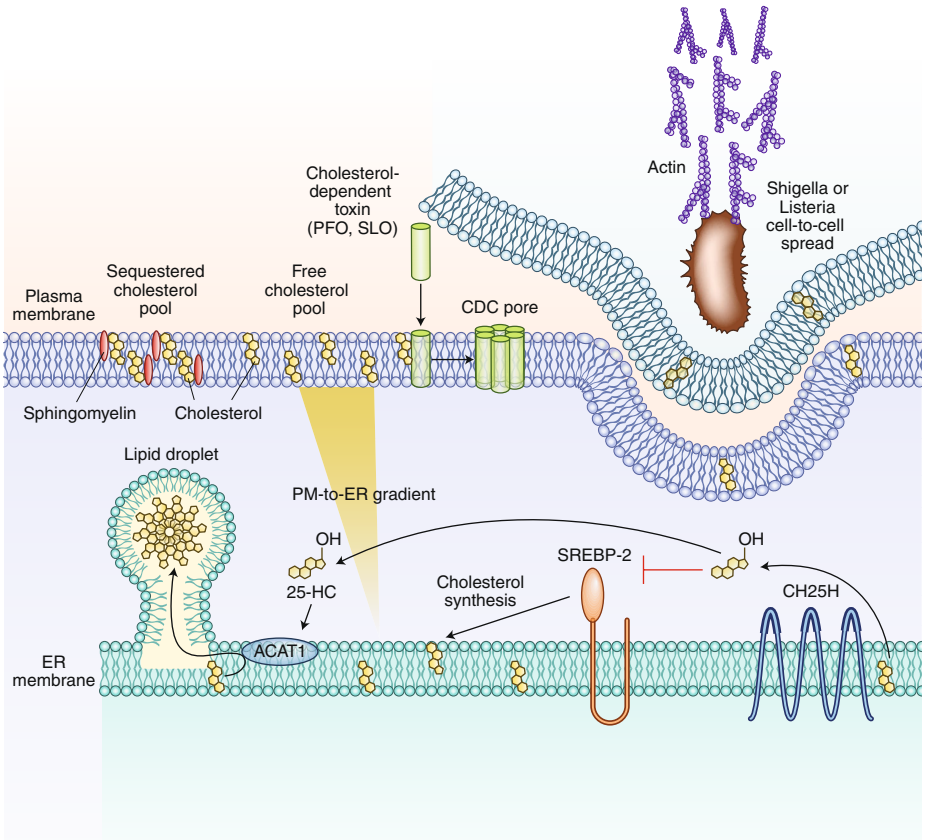

Fig. 1 | 25-HC blocks bacterial cell-to-cell spread and toxin activity via sequestration of plasma membrane cholesterol. Cholesterol in the plasma membrane (PM) is either sequestered via association with sphingomyelin or is accessible and able to exchange with cholesterol in the endoplasmic reticulum (ER). The cholesterol metabolite 25-hydroxycholesterol (25-HC) is generated by the interferon-induced enzyme cholesterol-25-hydroxylase(CH25H). 25-HC decreases ER cholesterol levels by activating ACAT-dependent esterification of cholesterol for storage in lipid droplets and by inhibiting cholesterol synthesis downstream of SREBP activation. The accessible pool of PM cholesterol is then depleted as it moves down a concentration gradient into the ER. Cells depleted of accessible cholesterol are resistant to cholesterol-dependent cytolysins (CDCs) and the cell-to-cell spread of bacterial pathogens such as *Listeria* or *Shigella*.

While the ER membrane cholesterol level is the primary signal that controls SREBP activation, cytokine signals provide an additional layer of control in the contexts of infection and inflammation. For example, treatment of macrophages with type I interferon (IFN) downregulates cholesterol biosynthesis[5]. Type I IFN signaling induces the expression of cholesterol-25-hydroxylase (CH25H), an ER-transmembrane enzyme that hydroxylates cholesterol on carbon-25 of its side chain, generating the oxysterol

25-HC. This oxysterol inhibits cholesterol biosynthesis by binding to Insig, causing it to trap the SCAP–SREBP complex in the ER. There is growing interest in understanding why myeloid cells actively repress their cholesterol biosynthesis following interferon stimulation. Recent studies have implicated 25-HC in regulating diverse immunological processes, such as IgA class switching[6], cytokine production[7,8], inflammasome regulation[9] and antiviral responses[10,11]. Interestingly, CH25H was identified in a cDNA library screen as providing protection against vesicular stomatitis virus infection when it was overexpressed in HEK293T cells in a non-cell-autonomous fashion[12]. It is now appreciated that 25-HC is released from CH25H-expressing cells, suggesting that type I interferon–activated myeloid cells may produce this oxysterol to protect neighboring cells from viral infection *in trans*.

To address the question of whether 25-HC might also provide protection against bacterial infection, Abrams et al.[2] employed a cell-based assay to search for *trans*-acting factors downstream of interferon that provided protection against bacterial infection. The authors first observed that supernatants from IFN-γ-stimulated macrophages contained a molecule that hindered replication of the intracellular bacterial pathogen *Listeria monocytogenes*. Using a similar approach to the studies identifying CH25H as a non-cell-autonomous antiviral factor[12], Abrams et al.[2] performed a cDNA overexpression screen to identify IFN-γ target genes that protected epithelial cell lines from *L. monocytogenes in trans*. CH25H was the top hit from their screen, and the authors subsequently showed that the antibacterial activity is due to the production of 25-HC. Abrams et al.[2] observed that 25-HC did not act via bacterial killing, prevention of phagocytosis/vacuolar escape or inhibition of intracellular replication; rather, 25-HC treatment seemed to prevent the cell-to-cell spread of *Listeria*, a process that utilizes so-called actin rockets, which enable a form of host-driven actin-based motility.

In their study, Zhou et al.[1] took a distinct approach, specifically focusing on a class of toxins produced by many bacteria called cholesterol-dependent cytolysins (CDCs). After pretreating macrophages with a variety of different stimuli, the authors found that treatment with type I IFN or IFN-γ protects cells from lysis by CDCs, including streptolysin O (SLO) and perfringolysin O (PFO). This protective effect of interferon treatment was lost in CH25H-deficient cells, and the authors linked this to the loss of 25-HC production.

This pair of studies suggests that 25-HC interferes with two very different steps in bacterial pathogenesis, yet surprisingly, they both converge on a common mechanism[1,2] (Fig. 1). Using specific membrane probes, both groups observed that 25-HC treatment rapidly decreases the accessible pool of PM cholesterol. The authors argue that 25-HC inhibits SREBP and promotes the activity of acyl-CoA:cholesterol acyltransferase (ACAT), an enzyme that esterifies ER cholesterol for storage in lipid droplets. Both groups conclude that the free, accessible PM cholesterol pool moves to the ER in response to an acute drop in ER cholesterol levels[1,2]. The high level of ACAT activity drives this movement by continually packaging ER cholesterol into lipid droplets until the free, accessible PM pool is depleted.

How do decreased levels of accessible cholesterol in the PM impact bacterial pathogenesis? In the case of CDC toxins, Zhou et al.[1] argue for a conceptually straightforward model, based on prior studies showing CDCs must bind to the free cholesterol pool to form membrane pores. This argument is strongly supported by both in vitro and in vivo experiments by the authors in which *Ch25h*-deficient mice showed exacerbated lesions in response to skin challenge with CDCs. However, 25-HC has pleiotropic activities in vivo[6–11]. It will be interesting to investigate whether the increased susceptibility to CDCs in vivo is indeed linked to changes in the free pool of PM cholesterol and how this influences disease in the more complicated setting of a bacterial infection. The study by Abrams et al.[2] raises the interesting question of how decreased accessible cholesterol in the PM blocks bacterial cell-to-cell spread. Importantly, *L. monocytogenes* utilizes its own CDC, listeriolysin O (LLO), to escape from the vacuolar compartment after phagocytosis, as well as from the double-membraned vacuole that results from cell-to-cell spread. Despite the findings of Zhou et al[1], the protective effects of 25-HC on the cell-to-cell spread of *Listeria* do not appear to be mediated by interference with the activity of LLO. It is not clear how LLO circumvents the effects of 25-HC, but one important difference between LLO and other CDCs is that LLO is active only at the low pH encountered in phagosomes and not at the PM[13]. One possible explanation is that 25-HC does not modulate phagosome membrane cholesterol because this pool is not in equilibrium with PM and ER cholesterol. Further confirming that 25-HC can block cell-to-cell spread by a mechanism independent of CDC inhibition, 25-HC also inhibited cell-to-cell spread of *Shigella flexneri*, which does not encode a CDC. This result suggests a more general effect of cholesterol levels on membrane fusion or invasion. Consistent with this notion, it has been observed that 25-HC treatment blocks initial viral fusion with the host cell membrane as part of its antiviral activity[10].

Collectively, Zhou et al.[1] and Abrams et al.[2] have discovered a new physiological mechanism whereby 25-HC secretion from IFN-stimulated cells decreases free PM cholesterol in neighboring cells and broadly prevents their infection by diverse pathogens. An area of interest for future studies will be dissecting the molecular basis for why free cholesterol is required for the membrane fusion and invasion events exploited by bacterial pathogens. Additionally, improved understanding of the cellular sources and targets of 25-HC could lead to the identification of key tissue niches that utilize this circuit. Finally, it will also be of interest to investigate the mechanisms by which pathogens may evade this 25-HC-mediated innate defense pathway. ❐

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