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How are STAT1 and cholesterol metabolism associated in antiviral responses?

Tomoh Matsumiya* and Tadaatsu Imaizumi
Department of Vascular Biology; Hirosaki University Graduate School of Medicine; Hirosaki, Japan

Sterol metabolites are known to be associated with immune responses. 25-Hydroxycholesterol (25HC) is a cholesterol metabolite that is produced by macrophages. The production of 25HC was reported to be TLR-dependent, indicating the involvement of 25HC in innate immunity. Now Blanc et al. show that macrophages synthesize 25HC to exert an antiviral effect. STAT1 was shown to be the most essential transcriptional factor involved in the induction of cholesterol 25-hydroxylase (Ch25h), the enzyme required to produce 25HC, indicating the importance of STAT1 in oxysterol-mediated innate immunity.

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*Correspondence to: Tomoh Matsumiya; Email: tomo1027@cc.hirosaki-u.ac.jp

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The daily diet is often foremost in our minds when we think about cholesterol. Doctors request that patients with heart disease try to lower their blood levels of low-density lipoprotein (LDL) cholesterol. Cholesterol is a unique type of fat that possesses a complicated chemical structure. It is an essential component of mammalian cell membranes; therefore, upon its synthesis in the liver, it must travel throughout the body. Because cholesterol cannot be dissolved in blood, transport proteins called lipoproteins carry cholesterol to the tissues as needed. So far, the word "cholesterol" has received a negative reputation because of low-density lipoprotein (LDL) cholesterol, which is regarded as a villain. Once LDL is oxidized by oxidative stress, it can travel directly within the subendothelial space, where it is taken up by accumulated macrophages. Further accumulations of these cholesterol-loaded macrophages (foam cells) eventually form an atherosclerotic plaque.

Oxysterols are oxidized derivatives of cholesterol that are short-lived intermediates in cholesterol excretion pathways; nevertheless, they have physiological roles as cholesterol transporters, hormone receptor ligands, or regulators of cholesterol homeostasis.[1] Nearly four decades ago, during early studies into the negative feedback of cholesterol biosynthesis, oxysterols such as 25-hydroxycholesterol (25HC) were shown to be more potent suppressors of cholesterol synthesis than cholesterol itself.[2,3] The sterol biosynthesis-related enzymes are regulated by transcription factors known as sterol responsive element binding proteins (SREBPs). Recently, reports from the Goldstein laboratory have clearly demonstrated how 25HC can reduce cholesterol biosynthesis by inhibiting the protein processing and translocation of SREBPs.[4,5] These findings suggest that oxysterols are multifunctional lipid mediators.

Vertebrate animals have developed innate and adaptive immune systems that provide defenses against microbial infections. The innate immune system is the first line of defense against invasions of foreign pathogens. When pathogens such as bacteria and viruses enter cells, they are sensed by pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs) and RIG-like receptors (RLRs), which activate downstream antimicrobial signaling pathways.[6]

Is there likely to be a close relationship between lipid metabolism and antiviral responses? Actually, lipid metabolism is essential in viral infections. The invasion of enveloped viruses into cells depends on the fusion of the viral membrane with

the target cell membrane.[7,8] Additionally, cholesterol depletion inhibits both viral infection[9] and replication.[10] Currently, we understand the importance of cholesterol in viral infections. A remaining question focuses on the effects of oxysterol. In 2009, Bauman et al. presented an interesting report in the journal *Proceedings of the National Academy of Sciences* that addressed this question.[11] 25HC is synthesized by cholesterol 25-hydroxylase (Ch25h). Bauman et al. initially found that Ch25h is upregulated through a TLR-dependent pathway and that TLR-dependent induction of Ch25h mRNA expression is observed in all murine tissues. Interestingly, the maximum expression levels of Ch25h are observed in tissues with resident macrophage populations (e.g., Kupffer cells in the liver and alveolar macrophages in the lung). An in vitro study, in which TLR4 was stimulated as it initially responds to bacterial infections, further demonstrated that macrophages can secret 25HC in response to TLR stimulation, which suggests that the macrophages are a major source of 25HC in TLR-mediated innate immune responses. Thus, a follow-up question might be: what about viral infections? The next year Park and Scott from Johns Hopkins University responded to this question[12] when they found that double-stranded RNA (a TLR3 ligand) and lipopolysaccharide (a TLR4 ligand) both could induce Ch25h expression to the same extent. Both the type I IFN receptor (IFNAR) and STAT1 were required for dsRNA-mediated Ch25h expression. In viral infections, TLR3 recognizes dsRNA and subsequently induces type I IFN expression. Hence, the authors claimed that Ch25h is an IFN-stimulated gene (ISG).

Oxysterol is likely to function in innate immunity as well as in the regulation of cholesterol biosynthesis. Is oxysterol either the *lapis philosophorum* (the philosopher's stone) or an evil agent in viral infections? Blanc et al. very recently reported a role for 25HC in antiviral immune responses (an overview of their report is illustrated in **Fig. 1**).[13] The researchers initially used a metabolomics analysis to investigate which types of oxysterols were secreted from macrophages in response to viral infections and found that only 25HC was produced and secreted. Consistent with this initial finding, the expression of Ch25h mRNA, which encodes the enzyme that converts cholesterol to 25HC, was induced in response to viral infection, type I IFN, or type II IFN. Moreover, IFN treatment significantly lowered the free cholesterol levels, suggesting a reduced biosynthetic output from which to generate additional metabolites. Importantly, other cholesterol hydroxylases such as Ch27h or cholesterol-7α-hydroxylase were not induced by viral infections or IFNs. Taken together, these findings suggest the importance of Ch25h in antiviral activity. Next, the authors asked whether 25HC could inhibit viral replication. Cells that were treated with 25HC inhibited mouse cytomegalovirus (MCMV) replication in a concentration-dependent fashion. The concentration of 25HC that was required to inhibit viral replication in macrophages was considerably higher than the concentration required to disrupt SREBP processing. Interestingly, 25HC exerted stronger antiviral effects in lipid-depleted conditions than in normal serum. Neither 19HC nor 7αHC, which did not inhibit SREBP, could repress viral infection. In contrast, both 27HC and 24HC inhibited MCMV replication; these oxysterols are also known to inhibit the SREBP pathway.[5] From these observations, the authors inferred the physiological relevance of lipid metabolism in antiviral effects, although only 25HC is secreted from macrophages in response to viral infections. 25HC also inhibited the replication of many viruses, including the influenza A virus (H1N1), herpes simplex virus-1 (HSV-1), varicella zoster virus (VZV), and murine gamma herpes virus 68 (MHV-68), yet had no inhibitory effects on the replication of adenoviruses. Therefore, 25HC is likely to be highly potent as an inhibitor of many types of viruses, with some exceptions. Conditioned media from virus-infected or IFN-treated macrophages possessed marked antiviral activities; however, this antiviral activity was partially abrogated in the presence of a 25HC-specific antagonist. Such experiments are important, as other antiviral factors have also been shown to participate in antiviral immunity.[14,15]

25HC can be further metabolized to other bioactive lipids; therefore, one might ask whether 25HC acts directly as an antiviral agent. The Epstein–Barr virus-induced gene 2 (EBI2) protein directs follicular B cell migration and localization.[16] 7α, 25-dihydroxycholesterol (OHC), which is converted from 25HC by cholesterol-7α hydroxylase, is known to be the natural ligand for EBI2.[17] However, this candidate has been excluded because both viral infections and IFN treatment inhibited EBI2 mRNA expression, while IFN accelerated the production of the 7α, 25-OHC precursor 25HC. Furthermore, an enantiomer of 25HC was shown to exert antiviral effects at a much higher concentration. Thus, the authors claim an essential role for 25HC in antiviral responses. Oxysterols such as 25HC or 27HC are known to be ligands for the liver X receptor (LXR).[18] Antiviral responses were shown to trigger the downregulation of SREBP2 target genes and the upregulation of LXR target genes; however, exogenous LXR ligands exhibited low levels of antiviral activity. Perhaps these findings indicate that LXR activation is insufficient to inhibit viral replication.

25HC-mediated SREBP inactivation results in the suppression of SREBP target genes that are involved in the mevalonate pathway. The mevalonate pathway is essential to generate isoprene chains for protein prenylation, and viruses have been shown to require prenylated viral or cellular proteins.[19] Blanc et al. previously reported upon the importance of geranylgeranyl transferase II-mediated prenylation in MCMV replication.[20] Next, Blanc et al. examined a possible role for 25HC in prenylation-dependent viral replication. Metabolic rescue experiments showed that isoprenoids could restore viral replication after a treatment with non-saturating inhibitory levels of 25HC. However, isoprenoids failed to rescue viral replication in the presence of saturating inhibitory levels of 25HC. These observations indicate that 25HC-mediated antiviral activity is partially dependent on the mevalonate pathway. The authors also indicated that further clarification of the role of 25HC in protein prenylation is required.

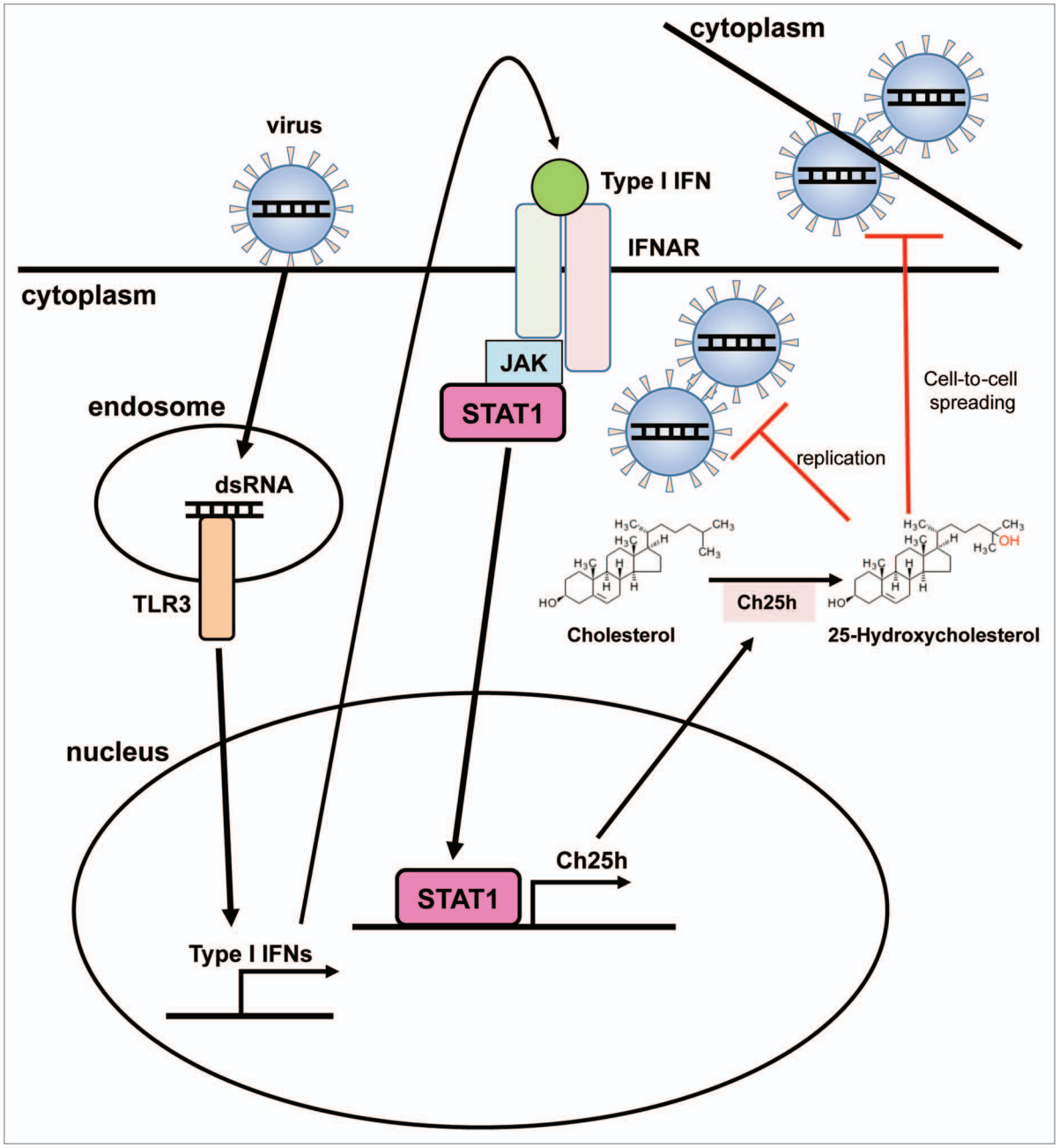

Figure 1. Schematic overview of 25HC-mediated antiviral activity. Upon viral infections in cells, exposed dsRNA is sensed by TLR3 and results in the expression of type I IFN. Secreted type I IFN subsequently binds to its receptor, IFNAR, on cell surfaces and activates the JAK-STAT1 signaling pathway. Blanc et al. discovered that STAT1 directly regulates the expression of Ch25h. 25HC, which is catalyzed by Ch25h, exerts antiviral activities such as the inhibition of viral replication and cell-to-cell spreading.

Viral infections are comprised of multiple steps that range from viral entry to the production of progeny virus particles. At what stage in the viral life cycle does 25HC exert its inhibitory effects? To explore this question, the authors performed a series of experiments and reported the following findings: 25HC failed to inhibit both MCMV viral entry and the number of primary infectious foci, markedly inhibited the cell-to-cell VZV spreading, did not alter cell viability rates, even at higher concentrations, had marked inhibitory effects on plaque development, inhibited the growth of both intracellular and secreted extracellular virus, reduced the levels of viral DNA replication and inhibited early and late-entry viral gene expression. These comprehensive data strongly suggest that 25HC inhibits viruses at the post-entry stage.

Park and Scott previously reported that TLR-mediated Ch25h expression is type I IFN- and STAT1-dependent.[12] However, exactly how IFN signaling, particularly STAT1, regulated Ch25h expression remained unknown. In the present study, the authors found that viral particles trigger PRR and subsequently induce Ch25h

expression. As shown previously by Park and Scott,[12] Ch25h expression in response to viral infections or IFNs was almost completely abrogated in STAT1-deficient macrophages. Ch25h expression was not observed in Tyk2-deficient macrophages in response to viral infections, which indicated the importance of the type I IFN receptor-meditated pathway in Ch25h expression. Finally, a promoter analysis and a ChIP assay revealed that STAT1 binds directly to the Ch25h promoter region.

IFNAR-JAK-STAT signaling is the cardinal pathway through which ISGs are induced in antiviral responses. To date, such ISGs have been shown to attack infected viruses directly. In the present study, Blanc et al. demonstrate the antiviral activity of 25HC, which was converted from cholesterol by Ch25h (an ISG). 25HC-mediated antiviral activity is LXR-independent and SREBP-dependent. Although cellular cholesterol biosynthesis has been shown to affect viral growth, many details concerning the contributions of SREBP-dependent and SREBP-independent pathways to 25HC-mediated antiviral activity remain to be elucidated. It will also be interesting to see the effects of RLRs on 25HC-mediated antiviral activity, because most non-immune cells, such as the airway epithelial cells or the intestinal cells, can detect viral RNA genomes through the RLRs.[6] In summary, this work provides new and important insights into a previously uncharacterized biological role for 25HC in antiviral innate immunity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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