Cholesterol 25-hydroxylase expression following Immune Activation in response to SARS-CoV-2 Infection

Editorial Comment

Jillian Davidson & Dino Rotondo
Strathclyde Institute of Pharmacy and Biomedical Sciences,
University of Strathclyde, Glasgow G4 0RE, Scotland, U.K.

Correspondence to D. Rotondo. Tel: +44 141 548 3629;
fax: +44 141 552 2562; e-mail: d.rotondo@strath.ac.uk

Lipid metabolism has long been recognised as a critical element in immune responses. Lipids play an integral role in both controlling immune responses via the biosynthesis of a very wide variety of mediators and also contribute to immune responses in metabolic roles including energy regulation and in membrane structure, particularly important for cell proliferation [1, 2]. There are numerous genes expressed which code for the many enzymes involved in key immune responses such as those in acute inflammatory activation. These enzymes include the expression of: phospholipases for the release of fatty acid precursors, cyclooxygenase for the production of prostanoids and ultimately the receptor proteins required to respond to the mediators. The expression is dynamically related to functionality when compared to constitutive proteins and can participate within a rapid time frame such as the suppression of pro-inflammatory cytokine production by newly expressed prostanoid receptors in acute inflammatory responses [3].

An often overlooked lipid pathway following immune activation is that of cholesterol synthesis. This is particularly important in providing cholesterol for membrane biosynthesis. The expression of genes in the cholesterol pathway can be modulated during immune activation. Indeed a commonly observed action is the upregulation of the cholesterol 25-hydroxylase gene (*CH25H*) following infection and it is a member of the Interferon-stimulated gene family. Viral stimulation or incubation of cells with the Toll-like receptor 3 (TLR 3) agonist Poly IC upregulates type I interferons and the downstream expression of a spectrum of genes including *CH25H* [4] and more recently specifically in the case of responses to SARS-CoV-2 [5]. This clearly indicates the importance of *CH25H* in antiviral responses. The function of cholesterol 25-hydroxylase (the *CH25H* product) is to hydroxylate cholesterol resulting in the formation of 25-hydroxycholesterol downstream of cholesterol biosynthesis and thus downregulates cholesterol production via the antagonism of sterol-response element binding proteins (SREBP) [6]. However, a critical aspect of the actions of 25-hydroxycholesterol is that it can suppress viral replication by inhibiting entry into cells and appears to do so by blocking the fusion of viruses with the plasma membrane [4].

A recent study in patients with confirmed SARS-CoV-2 infection shows greatly upregulated expression of the *CH25H* gene. The study of Wang et al. ▪▪[7] confirmed that type I

This is an accepted author manuscript of the following article: Davidson, J., & Rotondo, D. (2021). Cholesterol 25-hydroxylase expression following immune activation in response to SARS-CoV-2 infection. Current Opinion in Lipidology, 32(4), 266-267.
https://doi.org/10.1097/MOL.0000000000000765

interferons are upregulated along with the *CH25H* gene. This study correlated the expression of *CH25H* in macrophages obtained by bronchioalveolar lavage to disease severity. They observed that there was a greatly elevated expression in patients with moderate symptoms, however, the level of *CH25H* expression was very low in the macrophages from those patients with severe disease and actually lower than the level of expression in macrophages from healthy donors ▪▪ [7]. This would imply some failure of expression that could be related to a failure of protection. A particularly key aspect of this study that provides evidence for the protective action of *CH25H* expression is inclusion of experiments where they overexpressed *CH25H* and this resulted in decreased entry of SARS-CoV-2 virions into cells. In addition, they confirmed that the protective action was mimicked by 25-hydroxycholesterol which potently inhibited viral entry with an IC_{50} of 0.55 μM ▪▪[7]. This has been supported in another study that confirmed SARS-CoV-2 infection is suppressed by blocking membrane fusion to prevent viral release from infected cells ▪[8]. In addition, it has been long established that 25-hydroxycholesterol can suppress pro-inflammatory cytokine production and one of the first studies in this respect by Englund et al. ▪ [9] demonstrated that it could inhibit TNFα production in response LPS-stimulated macrophages. This indicates that 25-hydroxycholesterol could also protect against the major facet of severe SARS-CoV-2 infection i.e. the overwhelming inflammatory response leading to the worst severity and outcome which has recently been confirmed where incubation of macrophages (from severely ill SARS-CoV-2 patients) with 25-hydroxycholesterol results in a dramatic decrease in IL-1 production and also the suppression of a wide variety of pro-inflammatory genes [10].

It would appear that *CH25H* expression is protective in viral infections particularly with SARS-CoV-2 and provides a synergistic effect by inhibiting viral replication directly and also suppressing pro-inflammatory cytokine release. This strongly indicates that *CH25H* expression could be a primary prognostic marker to predict severity of disease and importantly, provide a therapeutic target for the development of 25-hydroxycholesterol analogues/ preparations in the effective treatment of severely ill patients.

Acknowledgements
None.

Conflicts of interest
The authors have no conflicts of interest to declare.

REFERENCES AND RECOMMENDED READING

[1] Batista-Gonzalez A, Vidal R, Criollo A, Carreno LJ. New Insights on the Role of Lipid Metabolism in the Metabolic Reprogramming of Macrophages. Front Immunol 2019; 10:2993
[2] Hubler MJ, Kennedy AJ. Role of lipids in the metabolism and activation of immune cells. J Nutr Biochem 2016; 34:1-7
[3] Kashmiry A, Tate R, Rotondo G, Davidson J, Rotondo D. The prostaglandin EP4 receptor is a master regulator of the expression of PGE2 receptors following inflammatory activation in human monocytic cells. Biochim Biophys Acta Mol Cell Biol Lipids 2018; 1863:1297-1304
[4] Liu SY, Aliyari R, Chikere K *et al.* Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. Immunity 2013; 38:92-105
[5] Wang R, Simoneau CR, Kulsuptrakul J *et al.* Genetic Screens Identify Host Factors for SARS-CoV-2 and Common Cold Coronaviruses. Cell 2021; 184:106-119 e14
[6] Holmes RS, Vandeberg JL, Cox LA. Genomics and proteomics of vertebrate cholesterol ester lipase (LIPA) and cholesterol 25-hydroxylase (CH25H). 3 Biotech 2011; 1:99-109
[7] Wang S, Li W, Hui H *et al.* Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and other coronaviruses by depleting membrane cholesterol. EMBO J 2020; 39:e106057
[8] Zang R, Case JB, Yutuc E *et al.* Cholesterol 25-hydroxylase suppresses SARS-CoV-2 replication by blocking membrane fusion. Proc Natl Acad Sci U S A 2020; 117:32105-32113
[9] Englund MC, Karlsson AL, Wiklund O, Bondjers G, Ohlsson BG. 25-hydroxycholesterol induces lipopolysaccharide-tolerance and decreases a lipopolysaccharide-induced TNF-alpha secretion in macrophages. Atherosclerosis 2001; 158:61-71
[10] Kim H, Lee HS, Ahn JH *et al.* Lung-selective 25-hydroxycholesterol nanotherapeutics as a suppressor of COVID-19-associated cytokine storm. Nano Today 2021; 38:101149

Annotated bibliography:

- Englund MC, Karlsson AL, Wiklund O, Bondjers G, Ohlsson BG. 25-hydroxycholesterol induces lipopolysaccharide-tolerance and decreases a lipopolysaccharide-induced TNF-alpha secretion in macrophages. Atherosclerosis 2001; 158:61-71
  This was one of the initial studies that clearly indicated the ability of 25-hydroxycholesterol to potently inhibit pro-inflammatory cytokine production.

- Wang S, Li W, Hui H *et al.* Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and other coronaviruses by depleting membrane cholesterol. EMBO J 2020; 39:e106057
  This study showed *CH25H* expression was very low in the macrophages from those patients with severe SARS-CoV-2 illness and that overexpression suppresses viral replication.

- Zang R, Case JB, Yutuc E *et al.* Cholesterol 25-hydroxylase suppresses SARS-CoV-2 replication by blocking membrane fusion. Proc Natl Acad Sci U S A 2020; 117:32105-32113
  This clearly confirms the mechanisms by which expression of cholesterol 25-hydroxylase via the production of 25-hydroxycholesterol and subsequent blocking of viral membrane fusion.