Ceramide-1 Phosphate: Multi-targets Immune Adjuvant for Controlling Covid-19 infection

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Abstract

Sphingolipids are amphipathic molecules and critical for the progression of various respiratory diseases. In this context we have recently demonstrated that Sphingosine 1 phosphate (S1P) is able to skew Interferon responses, inhibits IL-6 and pulmonary fibrosis and control M. tuberculosis infection in animal model. This led us to presume that this might control Covid 19 infection as well which utilize ACE-II receptor for its entry into the cells. Since activation of ACE-II receptor is associated with the activation of S1P Receptor 1 signaling and subsequent pulmonary fibrosis, cardio myopathy and Th17 responses which are the main reason of Covid19 mediated deaths. In view of this paradox, S-1P is not the right target for controlling Covid-19 infection. Therefore in such conditions other sphingolipids counterpart e.g. Ceramide 1 phosphate (C1P) can augment immunity and control Covid-19 infection by enhancing autophagy, adaptive immunity (Th1 programming) MHC-I dependent cytotoxic T lymphocytes (CTL) response

Introduction

Sphingolipids are dual specific lipids which favor both host and pathogens during acute / latent phase of respiratory infections (1). Sphingolipids and glycosphingolipids are integral part of plasma membrane and major constituents of fibroblast / epithelial cells that line the mucous membrane of the lungs forming an effective barrier. Additionally, glycosphingolipids are highly enriched in neurons, epithelial cells of skin and various organs and might contribute to the tropism, attachment, replication and pathogenicity of viruses targeting related organs (2;3). Recently, sphingolipids and glycosphingolipids have been detected in cells or tissues infected with variety of pathogenic viruses which exploit sphingolipids pathways for their benefit. Viruses exploit membranes and their components such as sphingolipids in all steps of their life cycle including attachment and membrane fusion, intracellular transport, replication, protein sorting and budding. Sphingolipids regulate trafficking, signaling, and play a crucial role on the life cycle of various virus e.g. influenza A virus and human immunodeficiency virus-1 (HIV-1) by interacting with their surface glycoprotein (4). Sphingolipids are major constituents of lipid rafts which are important site of early replication of hepatitis C virus (HCV). Strong evidence suggests that sphingolipids and glycosphingolipids play an intimate role in HCV replication in liver cells (5;6) Affinity-purified HCV particles are enriched in sphingomyelin suggesting that sphingolipids are integral components of HCV envelope. Study by Aizaki et al demonstrated that sphingomyelin facilitates HCV internalization, perhaps via fusion of the virus envelope with endocytic membrane to release HCV genome into the cytoplasm (7) Another compelling study documented the role of sphingomyelin in West Nile virus (WNV) and NPA replication (8;9) [8,9]. WNV replicates at a much higher level in

mice deficient in acid sphingomyelinase (unable to catabolize sphingomyelin). This suggested that sphingomyelin accumulation enhances WNV infectivity. Consistent with these findings, adding sphingomyelin to infected fibroblast cells markedly increased WNV infectivity (10). Further analysis showed that sphingomyelin colocalizes with WNV dsRNA at cytoplasmic foci, implying that sphingomyelin plays a role in the formation of the WNV replication platform. Interestingly pharmacological targeting of sphingomyelin synthesis (DS609 and SPK-601) markedly reduced the infectivity of WNV released from infected cells. These findings suggest that sphingomyelin is also required for WNV attachment, internalization, and/or virus–endosome fusion. Glycosphingolipids are known to facilitate binding / fusion of the HIV membrane with the host cell membrane (11;12). Hug et al. demonstrated that glucosylceramide-derived glycosphingolipids found on the target cell membrane are involved in the organization of gp120–gp41, CD4, and chemokine receptors (13;14) into a membrane fusion complex which facilitate the binding of HIV virus with CD4+ T lymphocytes. So far much of the information about the involvement of Sphingolipids comes from Influenza A virus infection model (15). Influenza A virus enters the cytoplasm via receptor-mediated endocytosis. Few reports suggest that sphingolipids metabolites are involved in influenza virus's genome replication. Seo et al. found that cells infected with influenza virus harbor increased levels of Sphingosine kinase (SK1), which phosphorylate free Sphingosine into Sphingosine 1-phosphate (S1P). The authors further showed that inhibition of SK1 impaired viral RNA synthesis and the subsequent nuclear export of newly generated vRNPs (16). Similarly, it was demonstrated that SK1 is critical for the nuclear export of viral proteins (NP, NS2, and M1) involved in transporting vRNPs from the nucleus to the cytoplasm. Later Tafesse et al. projected that perturbation

of host sphingomyelin biosynthesis inhibited the transport of influenza virus HA and NA to the cell surface, which in turn impaired viral maturation, budding, and release]. Influenza virus activates multiple signal transduction pathways (ERK pathways) to make the intracellular environment extremely affordable for viral propagation (17). SK inhibition was shown to interfere with NF-*κ*B pathway, which is essential for influenza viral RNA synthesis and the CRM1/RanBP3 nuclear export pathway which facilitates the transport of viral RNP complexes from the nucleus to the cytoplasm of the infected cell (18). Thus, SK inhibition seems to interfere with two very important stages in the intracellular life cycle of influenza virus. In same line, SK1 has been proposed to modulate the replication of other viruses. In addition to these reports, several viruses were shown to regulate the level or activity of SK1 enzyme. Like Influenza A virus, human cytomegalovirus (HCMV) increases SK1 activity and SK1 expression which contribute to the efficient virus replication. While blockade of SK1 expression decreased the expression of IE1 protein, over expression of same elevated the expression of IE1 proteins and virus particles (19). Similarly, Respiratory syncytial virus (RSV) increased the activity of SK1 and the mRNA expression of SK1 as well. Elevated expression of SK1 enhances RSV-induced activation of ERK MAPK and AKT signaling pathways which regulate the cell survival pathway upon infection. Novel Covid-19 virus interacts with human ACE-II receptor which facilitates the attachment and entry of this virus into epithelial cells (20;21). Interestingly these receptors are closely linked to S1P receptor 1 which maintains the barrier function on epithelial cells. Interestingly, activation of ACE-II receptor is accompanied with the activation of S1P Receptor 1 linked signaling which promote myopathy and subsequent fibrosis (22) in context of Covid-19 infection. Therefore, despite of anti-TB nature of S1P

(23), it is likely to rather promote Covid-19 infection. This is due to various allergic and autoimmune manifestations which are associated with S1P like degranulation of mast cells (24), tissue hypoxia, increased secretion of IL-6 and TGF beta contributing to aberrant pathology favoring Covid-19 replication. On these facts, S1P does not represent a right candidate for interventions and therapy benefit. On the basis of this, we strongly believe that other key Sphingolipids derivatives (Ceramide-1 Phosphate in particular) would afford immunity and control Covid-19 infection as mentioned in **Figure 1a** below

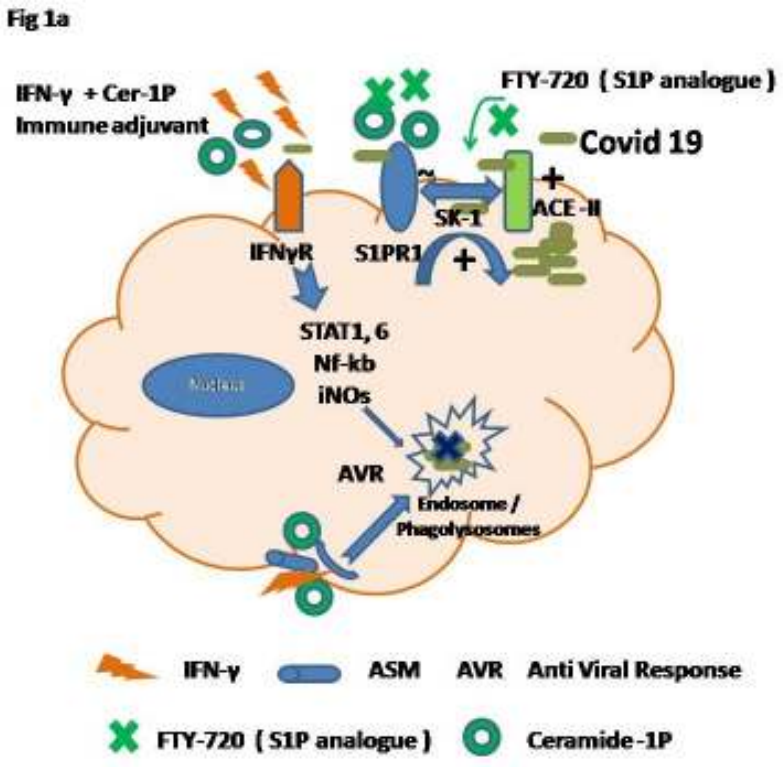

Figure 1 (a) Covid-19 viral entry in epithelial cells via ACE -II receptor is associated with S1P receptor 1. Activation of these receptors enhances permeability of the epithelium in Caveolin -1 / eNOS dependent manner. Internalization, activation of GPCR linked Sphingosine kinase 1 (SK-1) promote viral replication in lipid rafts and endosomal compartment. In such condition's stimulation of IFN gamma is a prerequisite for enhancing signaling favoring M1 / Th1 programming of macrophages and CTL. This can be augmented by promoting phosphorylation of membrane bound Ceramide to C-1P which is known to promote cell differentiation, autophagy and viral antigen presentation in MHC-I dependent manner. This is anticipated to contribute to anti-viral responses in epithelial / immune cells niches

Therefore, exploiting Ceramide / Ceramide 1 phosphate derivatives becomes paramount requirement for affording immunity against viral infection. Tweaking ceramide signaling in epithelial cell cultures is believed to hold therapeutics potential in controlling viral replication. This is due to immune adjuvant characteristics of Ceramides for activation of M1 effector macrophages and CD8+ effector T cells (25-27).

Major pharmacological perspective

Since, there are no specific therapeutic options available at present; there remains an urgent need for the discovery and development of Covid-19 specific antiviral therapeutics and vaccines. On the basis of association of ACE-II receptor with S1P signaling, employing FTY-720 for inhibiting S1P receptor 1 signaling seems to be an amicable approach for controlling Covid19 induced fibrosis. However it is difficult to predict whether this would bear therapeutic potential for controlling intracellular infection. In this scenario, it is highly advocated that enhanced de novo synthesis of Ceramide in the infected cells became a paramount requirement for inhibiting replication of Covid-19 virus and certainly warrants immediate investigation. Due to moderate to significant toxicity associated with purified ceramides, enhancing their do novo synthesis by pharmacological means is inevitable and safe approach for infusing Ceramide -1 Phosphate in the host by suitable pharmaceutical approaches. L-serine; an essential amino acid and precursor of Sphingolipids de novo biosynthesis, is believed to enhance the synthesis of ceramides -1 Phosphate. A recent study has demonstrated that L-serine promotes the synthesis of Ceramide in mouse embryonic fibroblasts (MEF), suggesting the important of ceramide (28) which are potent activators of resting macrophage to their effecter phenotype (29) Apart for this, L serine enhances uptake and utilization of glucose in iNOS+ M-1

committed macrophages for their immune defense against microbial challenge (30). Moreover L-serine derived Ceramide derivatives promote the proliferation of T and B cells [28] and enhance adaptive immunity against infection which is a pre-requisite for anti-viral responses. We strongly believe that enhancing ceramide contents would enhance autophagy, immunogenic signaling, enhance MHC-I dependent presentation of viral antigen to CD8+ T cells and overall adaptive immunity and afford clearance of Covid-19 infection. In view of these facts, repurposing drugs like Remedesvir, Ritonavir, Lopinavir and Favipiravir by crosslinking them with Ceramide-1 Phosphate is believed to enhance their efficacy against Covid-19 infection which is shown in **Figure 1b** below

Fig 1b

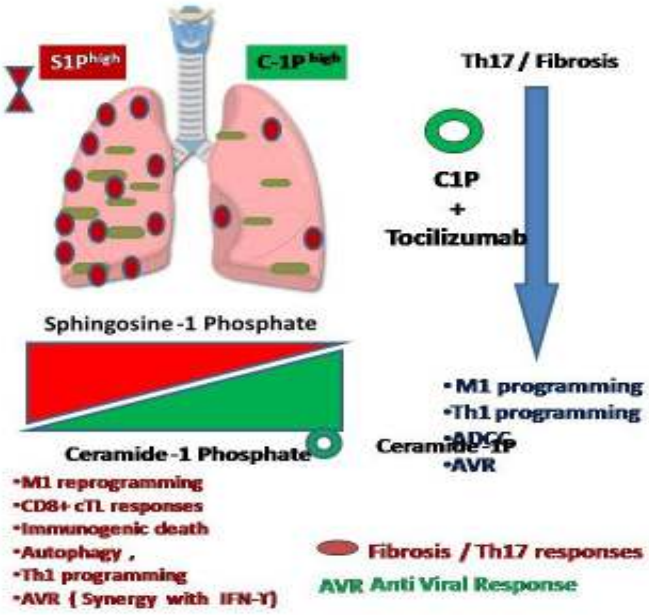

Figure 1 (a) Repurposing Tocilizumab by combining this with Ceramide-1 Phosphate can polarize Th17 response towards Th1 and mitigate fibrosis and help host in eradicating viral burden in lungs. High C-1P and low S1P rheostat is anticipated to promote M1/ Th1 programming inhibit pulmonary fibrosis and Th17 programming of lung which is pathogenic in nature and contribute to the infected related death. This will afford help immune system to inhibit replication of virus effectively and contribute to anti-Covid-19 responses.

In view of above facts, ceramide and its derivatives are anticipated to qualify both the pharmacological and immunological criteria of being introduced as potent adjuvant for existing retroviral therapies for the management of novel Covid-19 infection. This approach is certainly viable and has translational potential and deserves immediate attention by global scientific community.

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