**Phosphatidylserine Receptor Enhancement of SARS-CoV-2 Infection: AXL as a Therapeutic Target for COVID-19**

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**INTRODUCTION AND PURPOSE**

Therapeutics able to counter SARS-CoV-2 are currently limited. Desmethylavastatin finds utility with only the most critically ill, whereas convalescent sera and remdesivir have limited efficacy. Leveraging our understanding of RNA virus biology, we set out to identify host proteins that are exploited by SARS-CoV-2 for entry and identify efficacious inhibitors. Phosphatidylserine (PS) receptors, TIM1 and AXL, bind to virion- or apoptotic body-associated PS, internalizing cargo into the endosomal compartment. These receptors enhance entry of a wide range of enveloped viruses, including flaviviruses and influenzaviruses. We show here that SARS-CoV-2 also utilizes PS receptors to enhance entry. Further, an inhibitor extensively tested and shown to be safe in humans, bemcentinib, inhibits SARS-CoV-2 infection in vitro and in vivo models.

**METHODS**

Cells were treated with inhibitors as noted 1 hour before infection. Bemcentinib (BGB324) was used in these studies and passaged in VeroE6 cells. Presence of the viral genome was confirmed by RT-qPCR. Viral loads were measured 24 hours post-infection (hpi). Transfections used 50 ng human plasmid concentrations so that equivalent plasmid quantities were transfected. Furin cleavage site in our SARS-CoV-2 Spike/GAPDH ddCt was low, but not at higher hACE2 concentrations that 0.01. Presence of the viral genome was confirmed by RT-qPCR. Viral loads were measured 24 hours post-infection (hpi). Transfections used 50 ng human plasmid concentrations so that equivalent plasmid quantities were transfected.

**RESULTS**

AXL Knockout Impairs Infection + in-vivo Model

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As further assessment of AXL in SARS-CoV-2 infection, we repeated the above studies using the SARS-CoV-2 Spike/GAPDH ddCt. Viral loads were measured 24 hours post-infection. In the absence of AXL, SARS-CoV-2 viral load at 24 hours was suppressed at every MOI tested and bemcentinib had no antiviral effect on AXL KO cells. These data demonstrates the critical role of AXL during infection. Translating these observations to a mouse model of MfH (a related J CoV) treatment with bemcentinib by oral gavage significantly reduced infectious titers in liver at 5 dpi (G). Viral loads were similarly reduced in these experiments (not shown).

**CONCLUSIONS**

We identify that PS receptors (TIM1 and AXL) play major roles in the entry of SARS-CoV-2 virions. Our studies suggest that virus utilization of PS receptors internalizes virus through the endosomal compartment rather than at the plasma membrane. Inhibition of PS, signaling and cargo internalization by bemcentinib reduces infection as measured by viral loads and infectious foci. This inhibitory activity is specific to the entry stage of infection, and AXL knockout lung cells are far less permissive than wild type cells. This inhibitory activity was also observed in a coronavirus mouse model. In summary, PS receptors enhance SARS-CoV-2 infection and inhibition of AXL is a promising therapeutic target for COVID-19.

**ACKNOWLEDGEMENTS**

Experimental work was completed at the University of Iowa. Cell lines were provided by John Minna and Wendy Maury. Bemcentinib was provided by BerGenBio (Bergen, Norway). Meeting funding for Dana Bohan generously provided by the Immunology Graduate Program at the University of Iowa.