HCV and GBV-C NS5A protein inhibit TCR-mediated activation of human CD4+ T cells

Nirjal Bhattarai, Bennett E Thompson, James H McLinden, Jinhua Xiang, **Jack T Stapleton**
Iowa City Veterans Affairs Healthcare System and the University of Iowa, Iowa City, IA

ABSTRACT

Background: Hepatitis C virus (HCV) and GB virus C (GBV-C) are closely related members of the *Flaviviridae*, and both can establish persistent infection. HCV and GBV-C infections are characterized by reduced immune activation, delayed antibody response and altered T cell function. Although T cells are not the primary site of replication, HCV RNA is present in T cells and some evidence supports viral replication in T cells. We demonstrated that the GBV-C envelope protein E2 inhibits T cell receptor (TCR) signaling and contributes to reduced immune activation by competing for activation of the lymphocyte-specific protein tyrosine kinase (Lck). Here, HCV and GBV-C NS5A effects on T cell activation were studied.

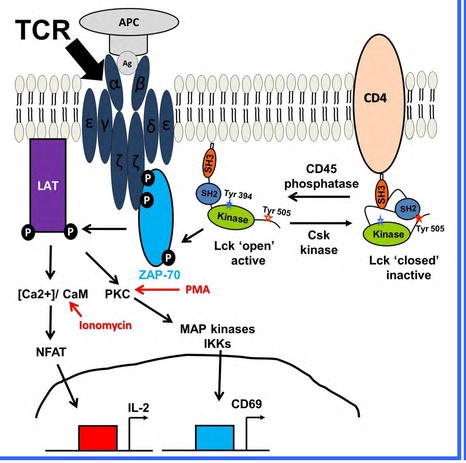
Methodology: Jurkat cells stably expressing HCV and GBV-C NS5A protein were studied. TCR signaling was monitored by IL-2 release (ELISA) and CD69 expression (flow cytometry) after stimulation with anti-CD3/CD28 or PMA-ionomycin (P+I). The MSD HCV NS5A inhibitor effects on TCR signaling was examined.

Results: T cell activation was inhibited in Jurkat cells expressing HCV or GBV-C NS5A following anti-CD3/soluble CD28 as compared to controls ($p<0.01$ for both IL-2 and CD69 regulation). Maintenance of cells in the MSD HCV NS5A inhibitor rescued HCV, but not GBV-C NS5A-mediated TCR inhibition following TCR stimulation ($IC_{50} \sim 2.5\mu M$). NS5A proteins inhibited TCR-signaling upstream of NFAT and PKC activation, as P+I was not inhibited by HCV or GBV-C NS5A.

Conclusions: TCR signaling pathways are modulated by HCV and GBV-C NS5A protein expression. The MSD HCV NS5A inhibitor blocked the TCR signaling defect induced by HCV, but not GBV-C NS5A, thus the inhibitor is specific for the HCV-NS5A protein, despite ~ 70% amino acid homology. Since, TCR activation is required for T cell function during viral infection; these data may provide insights into HCV and GBV-C pathogenesis and persistence.

Figure 1. Schematic of proximal T cell receptor signaling pathways.

Following TCR engagement, CD45 dephosphorylates Lck (Y505) exposing Y394, which is phosphorylated in *trans* by Lck. This activated Lck initiates the TCR cascade (ZAP70, LAT, etc.) resulting in T cell activation. Lck is phosphorylated at 505 by Csk. The sites of PMA and Ionamycin activation is downstream Lck and the TCR, and are shown bypassing TCR.



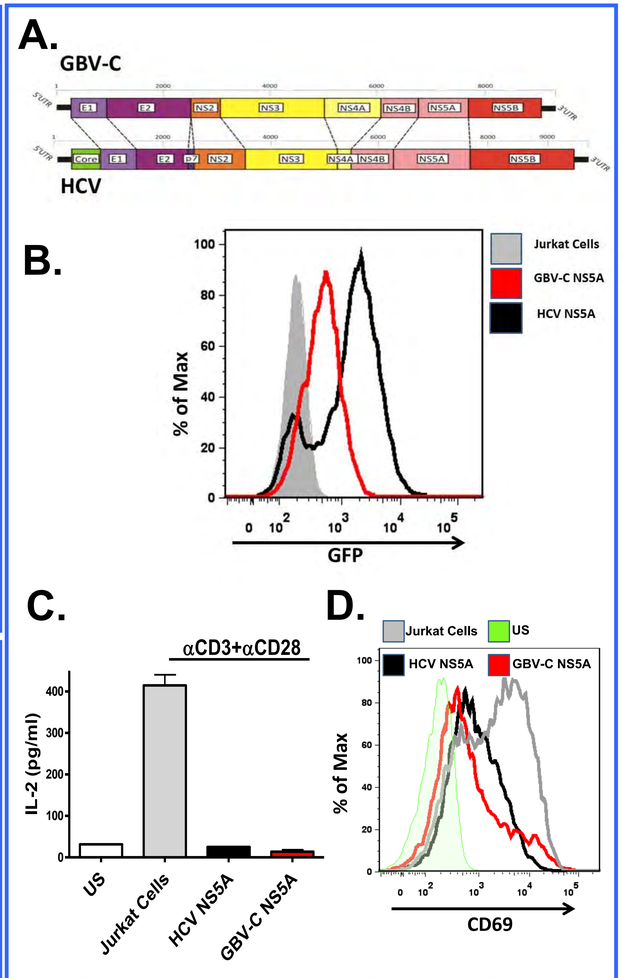


Figure 2: HCV and GBV-C NS5A inhibit TCR-induced T cell activation. GBV-C and HCV genome organization (A). HCV and GBV-C NS5A was expressed in Jurkat CD4+ T cells along with IRES GFP via a bi-citronic vector (B). HCV and GBV-C NS5A proteins inhibit TCR induced IL-2 release (C) and CD69 surface expression in Jurkat T cells following anti-CD3/CD28. US=unstimulated

Figure 3: Jurkat cells expressing HCV NS5A did not produce IL2 (Fig. 3) or upregulate CD69 (not shown) following TCR stimulation with anti-CD3/CD28. Activation downstream with PMA/Ionomycin overcame the block in IL-2 release

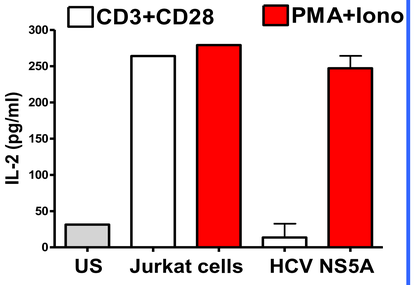


Figure 4: HCV NS5A regions involved in inhibition of TCR activation. HCV NS5A protein has three structured domains with an N-terminal amphopathic domain (A). Expresion of different HCV NS5A regions in Jurkat cells reveals a requirement for a proline-rich (SH3-binding) motif between domain II and III for Src kinase inhibition (B).

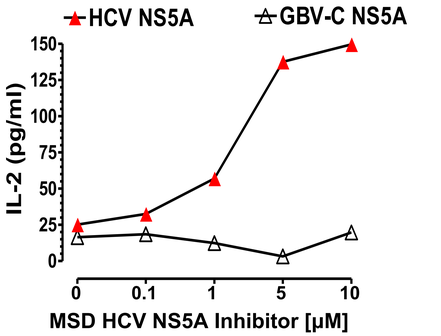


Figure 5: The effect of the MSD HCV NS5A inhibitor on T cell activation in cells expressing HCV NS5A or GBV-C NS5A inhibitor. The estimated IC50 for HCV was 1.8 μM.

Conclusions

- HCV and GBV-C NS5A proteins inhibit TCR-mediated activation of human T cells
- NS5A inhibits TCR signaling proximally, as PMA and ionomycin bypassed TCR-signaling to IL-2 and CD69 promoters.
- The HCV NS5A region required for inhibition includes a proline-rich domain between domain II and III that previously was shown to bind to SH3-domain of Src kinases.
- The MSD HCV NS5A inhibitor rescued HCV but not GBV-C NS5A-mediated inhibition of TCR signaling.

References

1. Terilli RR *et al.* Curr HIV/AIDS Rep. 10:51-8. 2013.
2. Xiang J *et al.* Proc Natl Acad Sci USA, 103:15570-15575, 2006.
3. Xiang J *et al.* J Immunol, 183:7860-9. 2009.
4. Bhattarai N *et al.* J Immunol. 189:2211-6. 2012.
5. Bhattarai N *et al.* J Immunol. 190: 6351-9.2013

Acknowledgements

This work was funded by Merit Review grants from the Department of Veterans Affairs (JTS and JX).