Does *H. pylori* prime human dendritic cells for *norovirus* infection?

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Abstract

Norovirus, a small, noneveloped RNA virus, is the most common cause of acute gastroenteritis in humans (Karst, et. al., 2014). Although pathogenic infection of intestinal epithelial cells in enteric disease is common, repeated attempts at infecting epithelial cells with human norovirus have been unsuccessful (Wobus et al., 2004). Research has shown that murine norovirus demonstrates replication in primary dendritic cells and macrophages (Wobus et al., 2004), and that it replicates remarkably well in STAT1 deficient mice, indicating that a similar system may be relevant in humans. Additionally, intestinal dendritic cells are required for extraintestinal spread of human virus in murine models (Karst, et. al, 2014). However, efforts to induce infection in human dendritic cells have been futile (Lay, et. al. 2010). Our hypothesis is that infection of dendritic cells with human norovirus is dependent on the presence of gastrointestinal stromal factors and signaling pathways, and that infection will occur primarily in STAT1 deficient cells. Previous research in this lab has concerned the identification of gastric stromal factors using gastric stroma- conditioned media, which is a model for the gastric microenvironment. We have thus confirmed SCM-derived immunoregulatory factors as a suitable model for generating dendritic cells with a mucosal phenotype. The proposed project will utilize both gastric and intestinal stroma-conditioned media, as well as *H. pylori* as a vehicle to suppress STAT1 signaling, to investigate whether mucosal stroma factors render human dendritic cells susceptible for infection with norovirus. This research applies to biomedicine in that it concerns the pathogenesis of a prominent human pathogen which is not well understood. Further understanding of this virus could allow for the development of an effective vaccine and more effective antiviral medication (Karst, et. al. 2004), as well as the identification of risk factors associated with coinfection.

INTRODUCTION

Norovirus is a genus that belongs to the family *Caliciviridae* (Lay, et. al. 2010). They are implicated in debilitating human gastroenteritis and have been found to chronically infect immunocompromised individuals (Karst et al. 2014). The genetic heterogeneity of the virus, and the appearance of new strains of virus every few years, makes it exceedingly difficult to target with a vaccine or antivirals (Karst, et. al. 2014). So, further understanding of the mechanisms of human norovirus infection is critical in developing better treatment.

Human norovirus is notoriously difficult to culture in vitro, so previous attempts to study the virus utilized animal model systems. However, a human B cell norovirus culture system has been developed recently (Jones et al, 2015). While this culture system is a great development, viral replication achieved in B cell culture systems is modest, and so *ex vivo* systems utilizing B cells still remain difficult to utilize.

Using a mouse model, researchers have determined that murine norovirus preferentially infects both bone-marrow derived and cell line antigen presenting cells (Lay et al. 2010), which suggests a similar mechanism may exist in humans. Additionally, *in vitro* B cell lines do not show an incredibly high rate of replication, suggesting that intestinal dendritic cells and macrophages could be the site of high level infection (Karst and Wobus, 2015), which is also evidenced in the murine model (Wobus et al. 2004).

Additionally, viral reproduction in murine STAT1 deficient cells was markedly higher than in the wildtype (Wobus et al. 2004), indicating that an individual who is immunocompromised would be at higher risk of prolonged infection. Norovirus is known to infect the intestine, but it is not known if it is a true pathogen of the stomach. A mechanism has been proposed that implicates *Helicobacter pylori* in gastric infection of norovirus. *H. pylori* has been shown to block STAT1 signaling pathways (Mitchell, Huynh et al. 2004, Wang, Chen et al. 2014), so a person infected with *H. pylori* would also be at higher risk of developing chronic norovirus infection. Additionally, a proposed mechanism of norovirus pathology implicates the motile microbiota in its transcytosis across the intestinal and gastric lining (Karst and Wobus, 2015). So, *H. pylori* infection could possibly exacerbate norovirus persistence through this mechanism as well.

METHODS

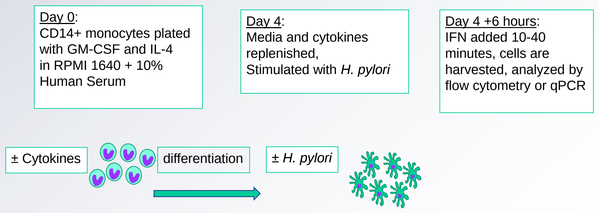
Cells: Human DCs were isolated using protocol published previously (Bimczok et. al, *Mucosal Immunol.* 2010 and 2014). CD14+ monocytes were isolated using MACS bead sorting and were cultured in RPMI1640 + 10% human serum with GM-CSF (25 ng/mL), and IL-4 (17ng/mL)) to generate MoDCs.

Culture: MoDCs were cultured in RPMI1640 +10% human serum at 37 ° C and 5% CO_2.

***H. Pylori* treatment:** Following three days incubation, MoDCs were activated with Helicobacter pylori in serum free broth at an MOI of 20.

IFN stimulation: Following H. pylori stimulation of 2-8 hours, cells were treated with 1-10 ng/ml IFN-gamma for 10-40 minutes, then fixed using 4% PFA.

Analysis: Cell differentiation and STAT1 phosphorylation was analyzed using flow cytometry and qPCR.



RESULTS

1. HuNoV detected in human fecal sample using qPCR and quantitated using a plasmid standard.

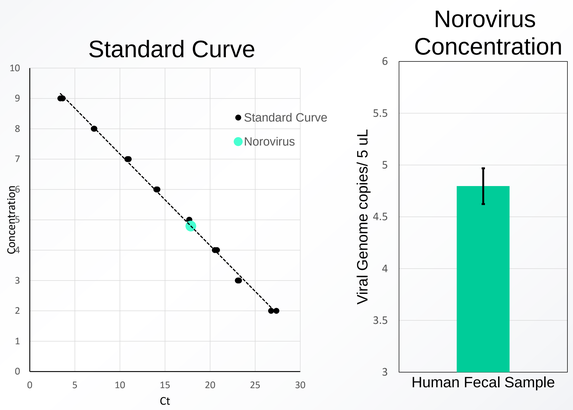
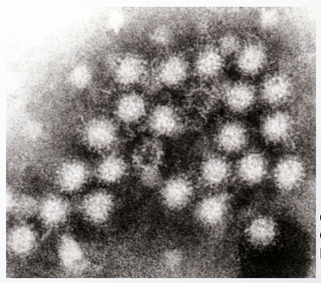


Figure 1a: Standard curve showing qPCR data ± SEM, where concentration values are *n* of plasmid concentration 1.55E^*n* copies/5uL. R^2=0.99 (n=2)



Courtesy Graham Beards, WIki

Figure 1b: TEM photo of norovirus in human fecal matter.

RESULTS cont.

2. H. pylori suppresses STAT1 signaling in DCs

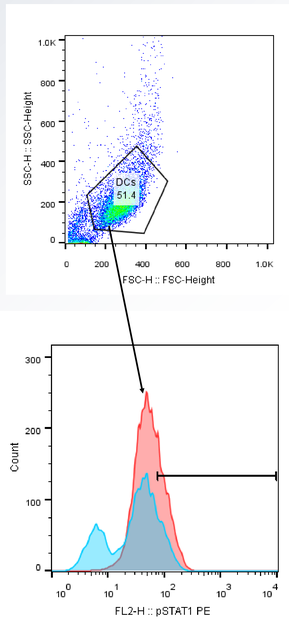


Figure 2a: FACS gating strategy for isolating dendritic cells

H. Pylori : 15.2 %
Medium only : 27.1 %

Figure 2b: FACS histogram showing percent of pSTAT1 after *H. pylori* stimulation

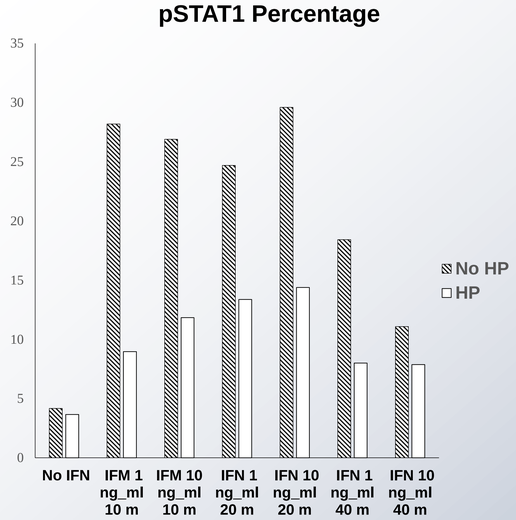
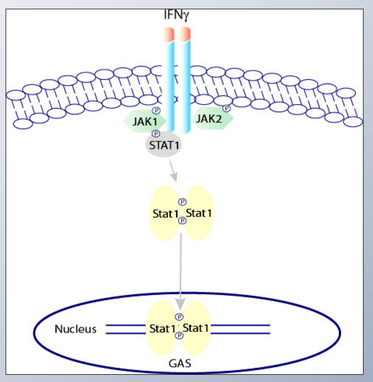


Figure 2c. FACS data showing percent pSTAT1, at different time points and IFN concentration, measured in percentage of pSTAT1 . (n=3)



Courtesy Isogen: Life Science

Figure 2d. Diagram of the STAT1 pathway in humans.

RESULTS cont.

4. STAT1 suppression is dependent on time post infection with H. pylroi

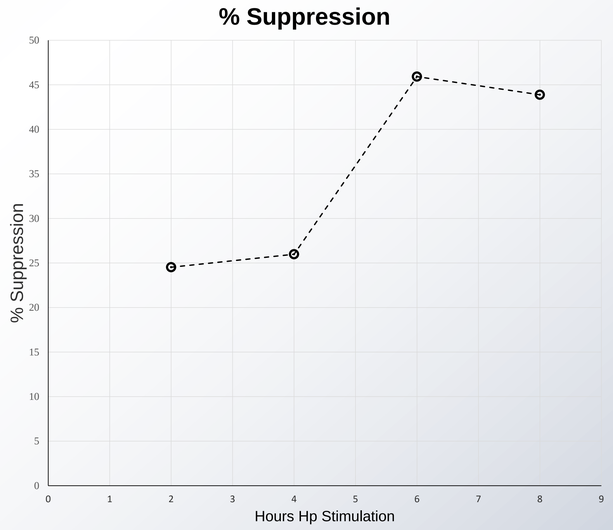


Figure 4: FACS data showing percent STAT1 suppression at 2, 4, 6, and 8 hours post *H. pylori* infection. (n=1)

CONCLUSIONS

***H. pylori* suppresses STAT1 phosphorylation in peripheral blood derived dendritic cells, primarily at hours 6-8 post infection.**

This suggests that an infection of dendritic cells with HuNoV would be more successful in a coinfection model, and that we have a window in which this would be successful.

Additionally, HuNoV detection is possible through the use of a Human GII plasmid and qPCR.

This enables us to successfully detect human norovirus in our samples.

FUTURE WORK

The next phase of this research will include DC infections with norovirus, as well as infection using gastric and intestinal S-CM.

This work has applications in public health, in that understanding the way that the virus interacts with the immune system will lead to better vaccines and more effective treatment.

ACKNOWLEDGMENTS

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