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A Model Method for Studying Norovirus

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Abstract
Norovirus is an extremely contagious positive sense RNA virus responsible for gastroenteritis. It has gained media attention due to its presence and impact on food and drinking water, and in the cruise ship industry. Researchers studying Norovirus have faced many challenges in their field with a limited number of reagents available for detecting the virus and growing it in culture, and no dependable small animal model. This research highlight focuses on the work of Taube et al. in the development of a mouse model for human Norovirus. This study used BALB/c mice deficient in recombination-activating genes 1 and 2 (Rag) and common gamma chain (γc) to demonstrate infection and replication of human Norovirus within the mice macrophages of the liver and spleen. They further showed the presence of human immune cells was not necessary to establish infection, but that the background strain of the mouse determined susceptibility. This discovery provides a huge step forward for basic and clinical applications of Norovirus research.

Keywords: gastroenteritis, mouse model, Norovirus

Introduction

Reliable animal models are essential for studying infectious agents. Different pathogens require specific host cell types and cell-specific gene expression that can vary from animal to animal in order to recapitulate their lifecycle *in vivo*. When available, mouse models often provide the best method for studying infection due to the ease of genetic manipulation, the quick reproduction rate, and the cost of housing. However, when pathogens cannot establish an efficient infection in mice, larger animal models or similar animal-specific pathogens are often utilized. While these models are useful to gain insight into the infectious agent in question, the development of mouse models of bacteria, viruses, and other pathogens often allow for the greatest manipulation and understanding of the disease lifecycle.

Research on human Norovirus (HuNoV), a positive sense RNA virus, has been hampered by the lack of a reliable small animal model of infection. This virus is responsible for sporadic gastroenteritis in children and adults, with limited diagnostic protocols or reliable laboratory research methods[1]. Owing to the lack of animal models, comparison studies using murine Norovirus are often employed. While these studies are useful, murine Norovirus has its limitations due to differences from HuNoV including dissimilar clinical manifestations, variations in host cellular receptors and susceptible cell types[2]. A recent article published by Taube *et al.,* in mBio aims to overcome these obstacles in a report demonstrating the first mouse model capable of supporting human Norovirus infection and replication.

Authors' results

Previous work in *in vitro* models and chimpanzees suggest that HuNoV may replicate in human immune cells[3-4]. Because of this, the authors' theorized that immune deficient mice transplanted with human immune cells (CD34+ hematopoietic stem cells) may be more susceptible to HuNoV infection. To test this theory, the researchers evaluated HuNoV infection in Balb/c wild-type (WT) mice compared to both Rag/γc double knockout mice

(Rag-/- γc-/-) and humanized Rag-/- γc-/- mice engrafted with human CD34+hematopoietic stem cells. Both HuNoV genogroups I and II were evaluated initially in these mice. Group II (GII), the most common serogroup associated with human outbreaks, was the only group detected in both mouse tissues and feces, and was used in subsequent experiments.

The researchers observed no change in virus genome numbers in Balb/c WT mice compared to the initial titers used for infection, denoting lack of HuNoV replication in immunocompetent mice. In Rag-/- γc-/- and humanized Rag-/- γc-/- mice there was an approximately 3 to 1,400 fold increase in viral titers, although there was no significant difference between these two groups. This demonstrated that HuNoV could replicate in immunodeficient mice independent of the presence of human immune cell engraftment. Together, these experiments demonstrated that an impaired immune response is necessary for HuNoV infection in mice, but the presence of human immune cells is not necessary to establish infection in mice.

To further evaluate this mouse model, Rag-/- γc-/- mice with either a Balb/c background or a commercially available Rag-/- γc-/- with the B6/B10 background were inoculated with HuNoV. In this comparison, there was a significant difference in viral titers in Balb/c mice compared to B6/B10 background strains, with Balb/c mice showing a significantly increased level of viral titers. Figure 1 shows representative data for each strain at 24 and 48 hours post-infection. These data highlight the importance of the host genetic background and immune system in mediating HuNoV infection. These differences could potentially promote further studies evaluating host-specific susceptibility factors to HuNoV and those necessary to establish efficient viral infection.

The extent of infection was characterized by immunohistochemical staining for HuNoV proteins. Using antibodies against VP1, NS4, and NS6, viral antigens were detected in the intestines, spleen, and Kupffer cells of Rag-/- γc-/- mice infected with HuNoV. Figure 2 demonstrates typical staining seen in liver and spleen cells of HuNoV infected mice. Staining with the anti-NS6 and VP1 antibodies showed similar patterns, with the morphology of positive cells in these tissues resembling to macrophages. Further staining of liver and spleen with the VP1 capsid antibody performed at 3 hours post-infection confirmed the viral antigens were from HuNoV expression, not residual inoculate virus. This staining, combined with genome quantification data, demonstrates that Rag-/- γc-/- mice with a Balb/c background are a working model of HuNoV infection in mice. The authors did note some limitations, including predominant detection of genogroup GII.4, indicating a need for future research evaluating strain dependence in this model.

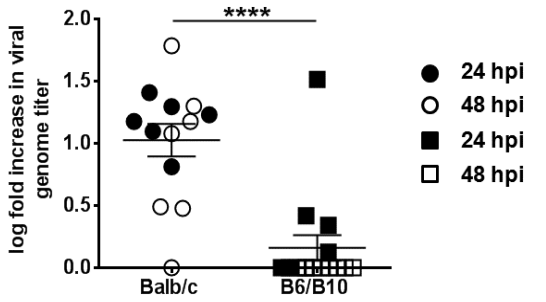

Figure 1. Differences in HuNoV titers dependent on genetic background. Rag-/- γc-/- mice with a Balb/c background showed a significant increase in viral titers compared to incolum when compared to B6/B10 background mice. *Images courtesy of Dr. C. Wobus and mBio.*

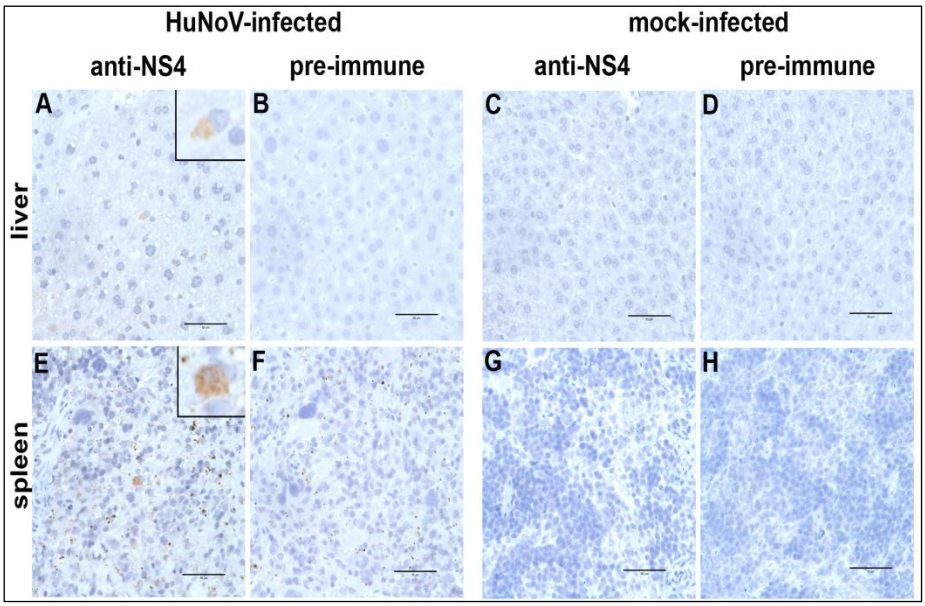

Figure 2. Immunohistochemical staining of HuNoV proteins in liver and spleen tissue. Tissue sections from HuNoV and mock-infected mice were incubated with anti-NS4 (A,C,E,G) or serum extracted prior to immunization (B,D,F,H). Specific NS4 staining was seen in HuNoV infected tissues in cells with macrophage-like morphology. *Images courtesy of Dr. C. Wobus and mBio.*

While fecal-oral transmission did not establish infection in intestine or extraintestinal site, inoculation by the intraperitoneal route allows for replication of HuNoV and sufficient infection and expression in relevant organs.

The implications of this mouse model are broad. By demonstrating efficient infection and replication, as well as identify target cells, this model can be applied to further studies evaluating HuNoV pathogenicity and host-pathogen interaction. Additionally, these studies could pave the road to identifying host susceptibility factors to HuNoV infections or possible vaccine target candidates. With further studies underway, this model provides an exciting leap for *in vivo* modeling of human Norovirus infection and replication.

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References

1. Glass, R.I., Parashar, U.D., Estes, M.K. 2009. Norovirus Gastroenteritis. *N Engl J Med*. 361: 1776-1785.
2. Tan, M., Jiang, X. 2010. Norovirus Gastroenteritis, Carbohydrate Receptors, and Animal Models. *PLoS Pathog.* 6(8): e1000983
3. Wobus, C.E., Karst S.M., Thackray, L.B., Chang, K.-O., Sosnovtsev, S.V., Belliot, G., Krug, A., Mackenzie, J.M., Green, K.Y., Virgin IV, H.W. 2004. Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS Biology*. 2(12): e432
4. Bok, K., Parra, G.I., Mitra, T., Abente, E., Shaver, C.K., Boon, D., Engle, R., Yu, C., Kapikian, A.Z., Sosnovtsev, S.V., Purcell, R.H., Green, K.Y. 2001.

Chimpanzees as an animal model for human norovirus infection and vaccine development. *PNAS*. 108(1): 325-330.

5. Taube, S., Kolawole, A.O., Höhne, M., Wilkinson, J.E., Handley, S.A., Perry, J.W., Thackray, L.B., Akkina, R., and Wobus, C.E. 2013. A Mouse Model for Human Norovirus. *mBio*. 4(4): 00450-13.