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Virology. Leaping the norovirus hurdle

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Bacteria and B cells solve the problem of studying human norovirus in the laboratory

Noroviruses are the leading cause of viral gastroenteritis worldwide with over 1 million hospitalizations and over 200,000 deaths per year (1). Outbreaks have been on the rise, particularly in hospitals, dormitories, and cruise ships. A major hurdle to understanding norovirus biology is the inability to culture human strains in the laboratory since the first human norovirus was identified over 40 years ago (2). On page XXX of this issue, Jones *et al.* (3) solve this problem. In discovering that human norovirus can infect B cells *in vitro*, the authors find that bacteria promote viral replication and may have been the missing factor.

Human norovirus has been very difficult to study. The cellular tropism is unknown, it cannot be grown in culture, and there is no robust small animal model. Much of our knowledge comes from human volunteer studies or experiments with individual viral components. The discovery of murine norovirus (4) provided a valuable tool because it replicates in mice and in cultured cells, but it is not known how closely murine norovirus mimics human norovirus.

The cellular targets of human noroviruses may include intestinal epithelial cells and immune cells, particularly B cells. For example, mice infected with murine norovirus have virus-positive cells in intestinal B cell zones, and mice lacking B cells have reduced viral titers (5-7).

Because human norovirus cannot be cultured, virus is acquired from stool of infected individuals. Standard practice is to filter the stool samples using 0.2-micron membranes to remove contaminating bacteria. However, Jones *et al.* observed that unfiltered stool samples contained norovirus that could replicate in cultured human B cells.

A hint to the identity of the missing factor came from a finding that human norovirus binds to *Enterobacter cloacae* bacteria (8). Certain strains of *E. cloacae* express histo-blood group antigens on their surface. These antigens constitute a family of glycans that are used in blood typing and are found in body fluids and on the surface of red blood cells, and epithelial cells of the digestive, respiratory, and genitourinary tracts. Human noroviruses bind to certain histo-blood group antigens, an interaction proposed to facilitate viral

attachment to cells (9). Indeed, individuals that lack certain histo-blood group antigens are resistant to human norovirus infection (10).

Jones *et al.* assessed whether the missing factor in filtered stool samples was bacteria expressing histo-blood group antigens. The authors demonstrated that human norovirus from filtered stool samples could infect B cells only when mixed with *E. cloacae* that express these antigens or synthetic H antigen, a specific histo-blood group antigen known to bind human noroviruses. Furthermore, exposure to *E. cloacae* or synthetic H antigen enhanced viral attachment to B cells, suggesting that bacteria expressing these antigens may promote viral replication by aiding attachment to host cells.

Comparing human norovirus and murine norovirus, Jones *et al.* examined whether bacteria promote murine norovirus infection *in vivo* and whether murine norovirus can infect B cells *in vitro*. They found that murine norovirus replication was reduced in antibiotic-treated mice, suggesting that bacteria promote viral infection *in vivo*. Additionally, they found that murine norovirus infects B cells *in vitro* and that this requires bacteria.

The discovery of Jones *et al.* underscores the importance of bacteria for enteric virus infection. To date, enteric viruses from four different viral families rely on bacteria for replication and/or transmission. Mouse mammary tumor virus (*Retroviridae*) binds to bacterial lipopolysaccharide, a molecule that induces immune tolerance (11). Reovirus (*Reoviridae*) requires the presence of intestinal bacteria for viral replication and pathogenesis (12). Poliovirus (*Picornaviridae*) requires bacteria for optimal viral replication and transmission, potentially through viral binding of bacterial surface polysaccharides such as lipopolysaccharide and peptidoglycan (12, 13). Thus, Jones *et al.* have added noroviruses (*Caliciviridae*) to this list of enteric viruses that benefit from bacteria. Validation of this promising new cell culture model for human norovirus infection will pave the way for studies that have been impossible for decades.

A common theme is emerging: Enteric viruses bind to bacterial surface glycans, which directly or indirectly promotes viral replication. There are several questions that emerge from the findings of Jones *et al.* relevant to this theme. It is not clear how bacterial histo-blood group antigens enhance human norovirus attachment to B cells. Studies on poliovirus suggest that bacterial surface glycans enhance the binding of virions to the viral receptor protein (13). Perhaps noroviruses that are bound to histo-blood group antigens on bacteria have enhanced attachment to a host cell protein (which may even be histo-blood group antigens). There is also the question of whether additional human norovirus genotypes infect B cells, and whether or not bacteria and/or bacterial and/or host histo-blood group antigens are required. There are more than 25 highly variable human norovirus genotypes. The binding site for histo-blood group antigens on viral particles is hypervariable and it is reasonable to expect differences in antigen binding affinity.

Do bacteria promote norovirus infections in humans? If so, how prevalent are bacteria that express histo-blood group antigens in the human intestine, and do some of those strains promote human norovirus infection more than others? Microbial communities vary from

person to person. It is possible that some individuals harbor more bacterial strains that promote human norovirus infection than others.

We are only beginning to understand the roles of commensal bacteria in the human gut for viral pathogenesis and/or transmission. Whether they are protective or preventive relationships should guide the use of treatments such as antibiotic-mediated microbiota depletion on enteric virus infection.

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