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Therapeutic Opportunities in Intestinal Microbiota–Virus Interactions

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The host microbiota has emerged a third player in interactions between hosts and viral pathogens. This opens new possibilities to use different tools to modulate the intestinal microbial composition, aimed at reducing the risk of or treating viral enteric infections.

Gut Microbiota Shape Enteric Virus Infection

Classically, virologists have considered viral infection a bidirectional (virus–host cell) process with no participation of external factors other than the immune system. However, this classical picture is changing in view of how some viruses exploit specific and direct interactions with the commensal microbiota from the mucosal niches they infect.

Accumulating evidence has demonstrated a key interaction between gut microbiota and intestinal viruses that leads to infection in mouse models. For example, the infection of mice by intestinal-replicating poliovirus [1] depended on the presence of intestinal bacteria. A similar situation has been recently described for the two viral groups responsible for the major percentage of acute gastroenteritis (AGE) worldwide: rotavirus (RV) and norovirus (NoV). RV infections are the leading cause of deaths due to AGE in children under the age of 5, while NoVs are associated with approximately 20% AGE episodes globally. Experiments in gnotobiotic models or animals with depleted intestinal microbiota have demonstrated the role of enteric bacteria in the infections of both viruses. Mice treated with antibiotics showed a decreased infectivity of murine RV [2], and this treatment also caused a similar effect in the murine NoV (MNoV) model [3]. Reinforcing this concept, it has been recently shown that the gut microbiota prompt MNoV replication through an antagonistic mechanism to interferon-lambda (IFN-λ) [4]. These facts conflict with the generally accepted role of the microbiota as a shield against pathogen infection, owing to their immunoregulatory functions and colonization-resistance effect (Box 1).

Recent results with human NoV (hNoV) also argue in favor of the microbiota's role in infectivity, although the existence of contradictory results indicates that more research is needed to have a clear picture of the mechanisms. While several cellular lines are available for infection by human RVs, it was not until recently that hNoVs were successfully replicated *in vitro* in B cells with the participation of the microbiota. The presence of gut commensal bacteria allowed hNoV infection in human lymphocytes, with the purified human blood group antigen (HBGA) substance H having the same effect: enhancement of hNoV attachment and replication [3]. HBGA-like substances expressed on the surface of certain enteric bacteria may be targets for viral attachment, and this has been demonstrated in some strains [5]. Many studies have correlated hNoV susceptibility with the secretor status (synthesis of H-antigen at mucosal sites dictated by a functional *FUT2* gene), and it has been recently demonstrated that secretor status also influences RV vaccine (RVV) immunogenicity [6]. The secretor phenotype has also been shown to impact intestinal microbial composition [7]. However, the hNoV tropism is still under discussion, and a recent *in vitro* hNoV replicating system has been set up that uses organoids derived from intestinal epithelial stem cells without a microbiota presence [8]. This has promoted a profound debate that has been further fueled by other conflicting results; although enteric bacteria such as *Enterobacter cloacae*, which expresses H-like antigens on its surface, enhanced *in vitro* hNoV B cells infectivity [3], the administration of *E. cloacae* in a gnotobiotic pig model antagonized NoV infection [9].

How Can the Gut Microbiota Be Manipulated to Fight against Enteric Viruses?

Currently, the role of microbiota in AGE remains elusive, but new applications beyond the state of the art are foreseen. Oral administration of classic members of the gut microbiota (e.g., *Lactobacillus* and *Bifidobacterium*) has proven beneficial in mitigating the severity of viral AGE. While this protective effect is mainly attributed to immunoregulation (e.g., enhancement of specific anti-RV IgA production) or to a simple competition for attachment to host cells (Box 1), the microbiota now appear as a 'double-edged sword' that can also promote infectivity of AGE-causing viruses. If the intestinal microbiota restrict infectivity but, in parallel, promote viral stability, attachment/entry, or act as a 'Trojan horse' that helps viruses reach their infection sites, then differences in the microbial composition could explain differences in viral susceptibility. Such differences were suggested to be responsible for the lack of RVV (an attenuated virus) efficacy in specific population groups. In a study conducted during a children RV vaccination program in Ghana, it was concluded that the intestinal microbiota of the population that positively responded to RVV were similar to those of age-matched European populations that have a high RVV response, whereas those of nonresponders differed substantially [10]. Furthermore, anti-hNoV

Box 1. Mechanisms of Intestinal Virus–Microbiota–Host interactions

Several mechanisms have been established or hypothesized on how intestinal viruses interact with the microbiota, influencing viral infectivity. Both promoting and antagonistic effects on infection are found (Figure I). The promoting mechanisms include:

- Virus binding to bacterial products (e.g., lipopolysaccharide or HBGA-like substances [15]) increases virion stability and protects it from physical stresses.
- hNoV-loaded bacteria could be transcytosed by intestinal epithelial cells (e.g., M cells from Peyer's patches), allowing the pass through the intestinal barrier and subsequent infection of immune cells (macrophages, dendritic cells, and B cells).

The antagonistic mechanisms include:

- Members of the microbiota specifically bind viruses, washing them out and impairing their binding to the intestinal epithelium [9].
- The microbiota–host crosstalk promotes immunoregulation, modulating the production of immune system molecules (e.g., IgA, IFN-β, and IFN-γ), which results in antiviral effects.

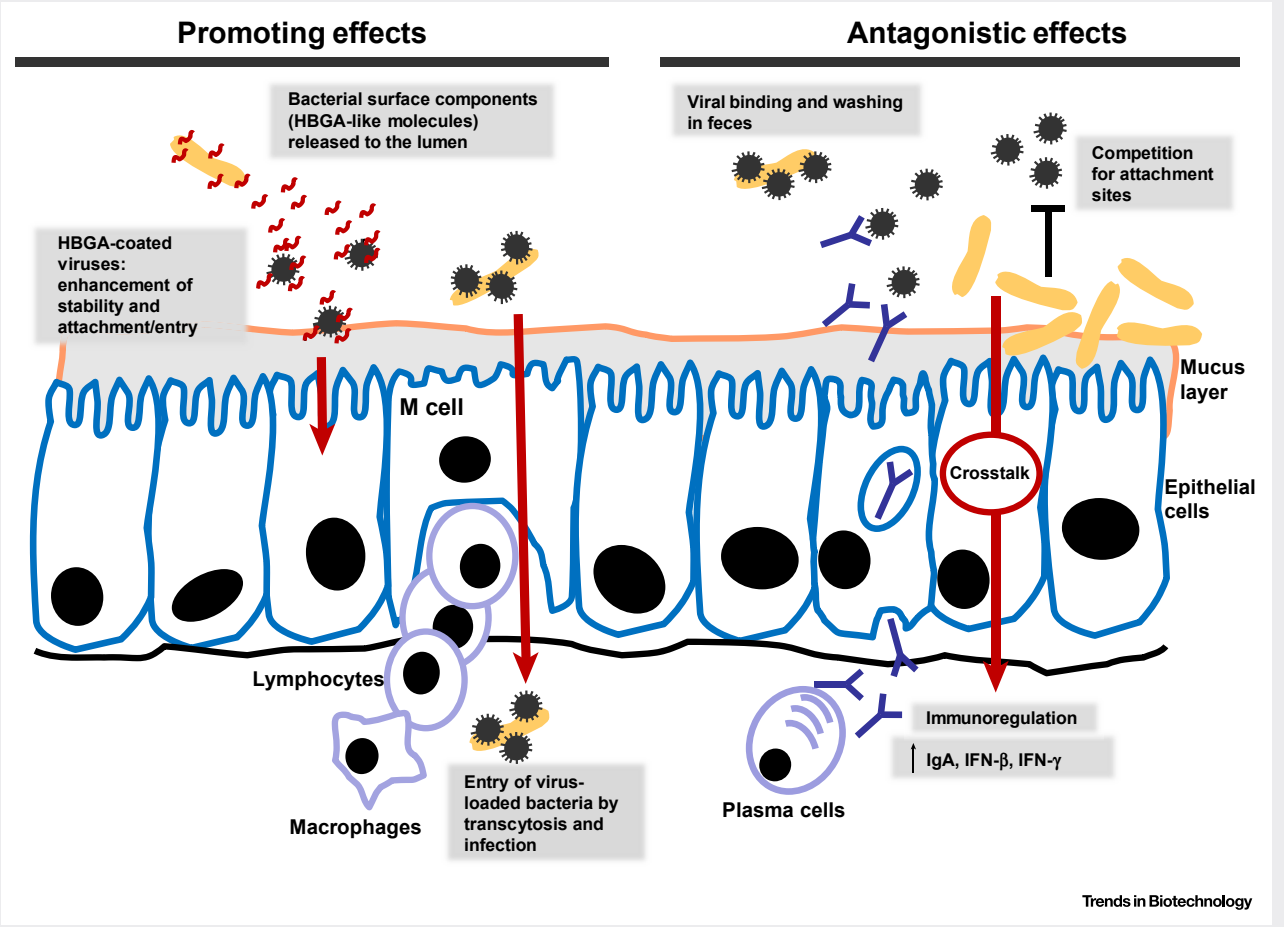

Figure I. Promoting and Antagonistic Effects of Microbiota in Acute Gastroenteritis. HBGA, human blood group antigen; IFN, interferon; Ig, immunoglobulin.

and anti-RV IgA levels in adults explained the differences in the intestinal microbial composition linked to the secretor (FUT2) status [7]. Currently, there is no commercially available vaccine for hNoV, and microbiota studies would be necessary to examine if the efficacy of a putative hNoV oral vaccine would also depend on the microbiota composition.

Remarkable gut microbiome and viral infectivity associations have been described in independent studies with European adults [7] and in the African RVV trial [10]. Thus, increased numbers of Bacteroidetes have been linked to the nonsecretor status (FUT2$^{-/-}$) in adults [7], while members of this phylum were also increased in children with low RVV response [10]. Furthermore, the higher presence of specific microbial taxa, such

as Ruminococcaceae, was linked to lower IgA titers to RV and hNoV in healthy adults. In parallel, higher proportions of *Ruminococcus* were detected in Ghanaian RVV nonresponders [10]. A negative correlation was also found for some specific anti-inflammatory bacterial species, such as *Faecalibacterium prausnitzii*, and hNoV susceptibility. Contrarily, others, such as *Akkermansia muciniphila*, were related to increased RV susceptibility [7], and *Streptococcus bovis* was present in higher numbers in RVV responders [10].

While these associations do not necessarily imply causality, host glycobiology, microbiota, and viral infectivity seem interconnected, and more research is needed to prove this theory and to discard the occurrence of confounders (e.g., age, diet, geographical location). Thus, studies in adults should be complemented with studies focused on children under the age of 5, particularly for RV, and with follow-up studies where the AGE incidence must be monitored. However, the finding of gut microbiota members as potential biomarkers of viral infectivity and/or risk of viral infection leads to a series of interesting questions that will probably lay the foundation for the development of new alternative therapies (Figure 1).

Would it be possible to increase the efficacy of oral vaccination by novel combinations of specific viral strains and bacteria? Positive correlations between microbiota/viral infectivity can be exploited. Specifically, microbiota analyses linked to the efficacy of vaccines (e.g., RVV) in different population settings [10] must be performed to identify candidate bacteria. Can antibiotics that target specific microbial groups be used to reduce the risk of RV and hNoV infection? Surprisingly, antibiotherapy appears as an alternative to fight viral AGE, although the risk–benefits of this approach must be considered. Could some of the identified biomarkers be used to counteract viral infection? These are anaerobic and fastidious bacteria; however, they are being proposed as new emerging probiotics. Dietary intervention strategies can also be envisaged. An intimate interrelationship between diet, immune system, and microbiota has been recognized when explaining risk and susceptibility to disease [11]. Diet has been described as the most powerful tool to modulate and shape gut microbiota, and diet intervention, including probiotics, prebiotics, and symbiotics, has been proposed for the treatment/prevention of microbiota-related diseases such as colorectal cancer, cardiovascular disease, obesity, and inflammatory bowel syndrome. While this still represents an unexplored field in virology, the recent anti-NoV effect of vitamin A supplementation has been explained through an increase in the *Lactobacillus* levels to modulate the microbiota, which results in IFN-β-mediated immunomodulation [12]. Fecal transplantation has been proven as another tool for modifying the gut microbiome, and it is useful for treating recalcitrant intestinal infections [13]. Although RV and hNoV cause self-limited AGE, the use of microbial cocktails or consortia for treating viral AGE through fecal transplantation can be anticipated.

Finally, the influence of the secretor phenotype on viral AGE inspires the idea of host mucosal glycosylation as a likely target for modulating RV/hNoV replication. The microbiota impact the mucosal glycosylation status by modulating the expression of host glycosyltransferases [14] and by providing a source of multiple glycosidases that act on the mucosa. If the microbiota's modification of the host glycans contributes to the infection process, either by promoting or limiting infection, this would provide a new repertory of therapeutic tools, including the use of specific glycosidases (purified enzymes

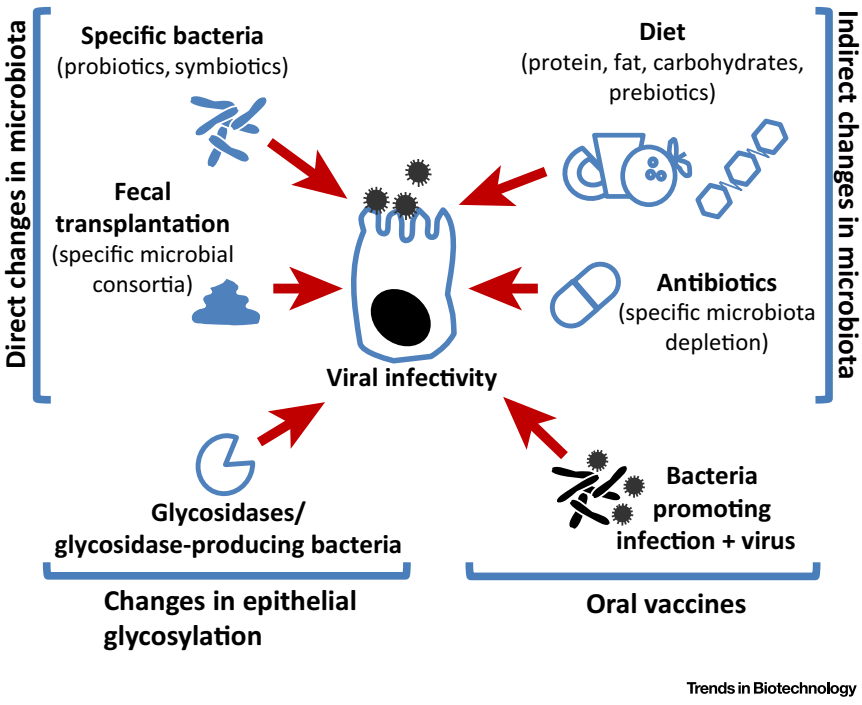

Figure 1. Possible Microbiota-Based Strategies for Antiviral Therapies. The different proposed strategies to manipulate the intestinal microbiota and modulate viral infectivity are depicted. Strategies include the promotion or direct use of particular bacteria for reduction of infectivity or the enhancement of the efficacy of infection for the development of more effective oral vaccines.

or glycosidase-expressing bacteria) to shape mucosal glycosylation and interfere with virus replication.

The virus–bacteria coevolution that has taken place over millions of years has established networks in the virus–host–microbiota triangle, where viruses exploit the microbiota and their related products to modulate some aspects of the infection process. Science remains far from establishing causal effects, and both direct and indirect effects may be present. As new mechanistic data on this triangular interplay are obtained, new opportunities will appear for therapeutic interventions and for viral preventive strategies.

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