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ADDENDUM

New perspectives regarding the antiviral effect of vitamin A on norovirus using modulation of gut microbiota

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ABSTRACT

Gut microbiota has been revealed to play an important role in various health conditions, and recent studies have suggested the modulation of gut microbiota as a therapeutic strategy. There is no effective treatment of norovirus infection, though vitamin A has been suggested to have an antiviral effect in an epidemiological study. We demonstrated that vitamin A significantly inhibited murine norovirus replication. Vitamin A supplementation significantly increased the abundance of *Lactobacillus* sp. during norovirus infection, which played a crucial role in antiviral efficacy, inhibiting murine norovirus. Therefore, we elaborated the antiviral effect of vitamin A via modulation of gut microbiota. Furthermore, we suggest a novel strategy, using potential probiotics, as having a protective and therapeutic effect on noroviral infection.

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Introduction

The gut microbiota plays an important role in energy and immune homeostasis of the gastrointestinal tract.[1-3] Recent studies have revealed that the modulation of gut microbiota significantly impacts various diseases including infectious diseases.[4,5] For example, fecal microbial transplantation (FMT), as a gut microbiota-targeted therapy, for the treatment of *Clostridium difficile*, could be a good example of the application of gut microbiota modulation for preventing infectious diseases.[6] In our previous study and other recent studies, metabolic improvement by commonly used drugs such as metformin, accompanied by modulation of the abundance of bacterial taxa (e.g, *Akkermansia muciniphila*), had a beneficial effect on obesity and type 2 diabetes.[7,8] In addition, gut microbiota influences healthy immunity from viral infections such as influenza virus and hepatitis virus.[9,10]

Norovirus is the most common cause of acute gastroenteritis, with clinical symptoms that include diarrhea, vomiting, nausea, abdominal pain, and fever lasting one to 3 d.[11] Unfortunately, there is no current treatment or vaccine effective against norovirus infection. A recent study reported that norovirus infection did not significantly alter the gut microbiota, the composition of gut microbiota was not considered in norovirus pathogenesis.[12]

In previous epidemiological studies, the norovirus infection rate and clinical symptoms decreased significantly with sufficient vitamin A supplementation.[13] Retinoic acid, the metabolite of dietary vitamin A, plays an essential role in innate immune response against viral infection. A recent study reported that sufficient vitamin A supplementation reduced both mortality and morbidity related to infectious gastrointestinal diseases.[14] Moreover, retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation-associated gene 5 (MDA5) signaling contribute to antiviral responses by activating type I interferons (IFNs).[15] Previous studies have revealed that MDA5- and STAT-1-deficient mice had a defect in their immune response to murine norovirus.[16,17]

In the current study, we investigated the possibility that gut microbiota, modulated by vitamin A during norovirus infection, could have a potential role in the treatment of, and preventive measures against, norovirus. Our findings provide new perspectives on how vitamin A exerts an antiviral effect against norovirus,

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and indicate a novel therapeutic strategy for the development of antiviral agents through gut microbiota modulation.

Inhibitory effect of vitamin A on murine norovirus replication

Vitamin A administration was considered to prevent norovirus infection and improve the clinical symptoms.[13] However, there has been no further study to evaluate the antiviral mechanism of vitamin A related to the control of norovirus infection. In our study, we showed that vitamin A effectively inhibited murine norovirus (Murine norovirus 1) replication *in vitro* and *in vivo*, providing evidence of the antiviral effect of vitamin A on norovirus. Further, we identified that IFN-β expression played a key role in immune responses, with vitamin A treatment. Various other studies revealed that type I and type II interferon signaling play a crucial role in antiviral immune responses.[15] Jung et al. reported that simvastatin increased the replication of norovirus, indicating that IFN-α has potential as an antiviral therapy.[18] Moreover, IFN-γ increased significantly in norovirus-associated diarrhea specimens, and IFN-λ determined the persistence of murine norovirus infection.[19,20] In addition, RIG-1 signaling was mediated by antiviral immune responses, stimulated by vitamin A, against murine norovirus, but the antiviral effect of *Lactobacillus* sp was not RIG-1-dependent.

Modulation of gut microbiota by vitamin A and murine norovirus

Recent studies have indicated that norovirus replication was related to the intestinal environment, including gut microbiota.[19,21] *Enterobacter cloacae*, a common bacteria identified in gut, was reported to facilitate norovirus infection.[21] On the other hand, disruption of gut microbiota by antibiotic treatment prevented the norovirus infection and persistence.[19,21] Ettayebi et al. successfully cultivated human norovirus in stem cells derived from human intestinal enteroid, a cultivation system requiring bile which is regulated by the intestinal microbiota.[22] Moreover, Kernbauer et al. suggested that murine norovirus can replace the beneficial function of bacterial microbiota.[23] These results strongly support our results that the modulation of gut microbiota is related to the inhibition of murine norovirus.

In our study, we characterized the gut microbiome by vitamin A administration and murine norovirus infection: 103 genera of bacteria were identified in the murine norovirus-infected mouse group during vitamin A administration, and 75 were also found in other groups. The result of principal coordinates analysis (PCoA) revealed that bacterial communities were more clearly clustered in weighted UniFrac than unweighted. Therefore, we expected that the abundance of certain bacterial taxa play an important role in the antiviral effect of vitamin A against murine norovirus infection.

The composition of the gut microbiome was changed significantly with murine norovirus infection in ICR mice, unlike the situation with Swiss Webster and inbred C57BL/6 mice.[12] Murine norovirus infection significantly increased the abundance of genus *Alistipes, Bacteroides, Bilophila, Corynebacterium, Desulfovibrio, Facklamia, Jeotgalicoccus, LE30, Oligella, Parabacteroides*, and *Yaniella*. On the other hand, genus *Dialister, Lactobacillus*, and *Veillonella* decreased with murine norovirus infection. And, vitamin A administration significantly increased the abundance of genus *Aggregatibacter, Allobaculum, Bifidobacterium, Dialister*, and *Enhydrobacter*.

Most of the identified bacteria have not been studied closely until now. Among them, we observed 2 genera, *Lactobacillus* and *Bifidobacterium*, which are well-known potential beneficial microbes. First, the abundance of *Lactobacillus* sp decreased significantly with murine norovirus infection, whereas it recovered during vitamin A administration. In recent studies, *Lactobacillus* sp showed an antiviral effect on various viruses such as rotavirus and influenza A virus mediated with IFNs signaling.[24,25] We also showed that the expression of IFN-β and -γ increased significantly by treatment of *Lactobacillus* sp in murine norovirus-infected RAW264.7 cells; IFN-β was remarkable. Therefore, the activation of IFNs by vitamin A and increased proportion of *Lactobacillus* sp plays a crucial role in the antiviral immune response against murine norovirus infection.

Putative probiotic candidates using modulation of gut microbiota

A recent study reported an antiviral effect on murine norovirus replication using *Lactobacillus paracasei* harboring nucleic acid-hydrolyzing 3D8 scFv.[26] In our

study, *Lactobacillus* sp were suggested as preventive probiotics against murine norovirus. Interestingly, the abundance of *Lactobacillus* sp increased significantly after murine norovirus infection during vitamin A administration. Various beneficial effects of supplementation with *Lactobacillus* have been reported in clinical settings.[27-29]

The genus *Lactobacillus* currently contains over 180 species and the effect of *Lactobacillus* sp is likely to be strain-dependent.[30,31] Among them, certain *Lactobacillus* sp have demonstrated antiviral effects against other viral infections such as rotavirus and human papillomavirus.[32] Although, we did no clearly identified *Lactobacillus* sp in fecal material, the development of potential *Lactobacillus* probiotics to prevent noroviral infection needs to be studied further, with the inclusion of clinical trials.

Further study

Firstly, the mechanism by which the abundance of *Lactobacillus* sp in gut microbiota is modulated by vitamin A should be identified to understand the inhibition of murine norovirus by vitamin A. We showed a correlation between *Lactobacillus* sp, vitamin A, and murine norovirus that supports the anti-noroviral effect of modulating gut microbiota via vitamin A administration. However, that correlation is not sufficient to confirm the inhibitory effect of vitamin A on norovirus. Based on Koch's postulates, *Lactobacillus* sp isolated from the modulated gut microbiota after vitamin A administration should be tested for inhibition of murine norovirus. Such antiviral immune responses by modulated gut microbiota have not been identified in previous research. Fecal microbiota transplantation using gut microbiota modulated by vitamin A could provide useful information to elucidate the antiviral effect of murine norovirus inhibition.

Secondly, we could investigate the antiviral effect of vitamin A on murine norovirus independently of the modulation of gut microbiota. Although activation of IFNs by RIG-1 and MDA5 signaling plays a key role in antiviral effects against a variety of different viruses, an increase in expression levels of those genes was not observed during inhibition of murine norovirus by *Lactobacillus* sp[15] Therefore, we could also investigate whether specific chemokines, such as chemokine (C-C motif) ligand 2 (CCL2) and interleukin 8 (IL-8), are involved in the immune response against murine norovirus infection.

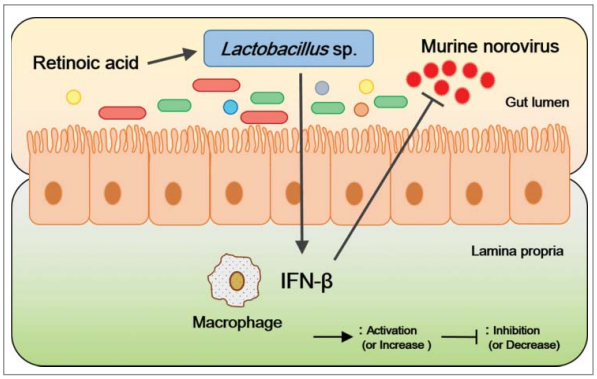

Figure 1. Model of murine norovirus inhibition by vitamin A. In this study, the abundance of *Lactobacillus* sp was considered to inhibit the murine norovirus proliferation via the upregulation of IFN-β; RA administration increased their abundance levels.

Finally, murine norovirus was used as a surrogate for human norovirus due to the lack of a cell culture infection model for human norovirus. Recently, there has been the remarkable research development of the replication of human norovirus using stem cell-derived human enteroids.[22] Therefore, the antiviral effect of vitamin A via modulation of gut microbiota could be better validated using this model for human norovirus in further studies.

Concluding remarks

We first identified that vitamin A administration effectively inhibited murine norovirus replication *in vitro* and *in vivo*. Moreover, vitamin A modulated the composition of gut microbiota; the increase in the relative abundance of *Lactobacillus* sp was remarkable after murine norovirus infection during vitamin A administration. Finally, we demonstrated the antiviral efficacy of *Lactobacillus* sp from *in vitro* experiments of the inhibitory effect of *Lactobacillus* treatment on murine norovirus replication; IFN-β signaling was involved in antiviral immune response (Fig. 1). Although the identified *Lactobacillus* sp should be evaluated further in human, we expect that a remarkable antiviral effect on norovirus is highly likely with these potential probiotics. Furthermore, the modulation of gut microbiota could be used as a novel strategy to control various viral infectious diseases.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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