Influence of probiotic Lactobacilli colonization on neonatal B cell responses in a neonatal gnotobiotic pig model of rotavirus infection and disease
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ABSTRACT
Colonization of the intestine with commensal bacteria is hypothesized to drive maturation of neonatal immune system. Some strains of lactic acid bacteria (LAB) may enhance protection against viral diarrhea in neonates; however, mechanisms are undefined. Our goal was to define the impact of intestinal colonization of gnotobiotic (Gn) pigs with probiotic LAB on development of intestinal and systemic B cell responses, including rotavirus (RV) specific, bacterial peptidoglycan (PGN) specific and total B cell responses. Gn pigs were fed mixed Lactobacillus acidophilus and L. reuteri and were inoculated with virulent RV (LAB+RV+). Pigs given RV only (LAB+RV+) or LAB only (LAB+RV-) were included as controls. RV infection induced similar RV-specific B cell responses in pigs with or without LAB, as indicated by similar profiles of serum and intestinal isotype-specific antibodies to RV and similar numbers of antibody secreting cells in the LAB+RV+ and LAB+RV- pigs. LAB alone induced significantly weaker B cell responses than RV alone as indicated by significantly lower total IgM and IgA titers in serum and small intestinal contents. Fecal LAB counts were significantly higher in the LAB+RV+ pigs compared to LAB+RV- pigs, which concurred with significantly enhanced PGN-specific serum IgA antibody responses; however, LAB did not significantly reduce RV shedding or diarrhea. Our findings demonstrated that germ-free pigs developed similar magnitude of virus-specific B cell responses to RV infection as those of the pigs colonized with commensal bacteria. However LAB colonization alone was not as efficient in promoting development of B cell compartment as RV infection in neonatal pigs.

INTRODUCTION
Earlier studies comparing Gn animals with conventional animals showed that Gn animals have less developed lymphoid tissues, with fewer IgA antibody-secreting cells and intraepithelial lymphocytes in the intestinal mucosa and a lower level of systemic immunoglobulin (Ig) when compared with animals that have a conventional indigenous microflora (1, 3). However, these observations were from Gn animals that were not exposed to pathogens. Enteric virus infections, such as RV infection of neonatal Gn pigs have been shown to significantly stimulate not only the development of RV-specific antibody-secreting cell (ASC) responses but also non-specific Ig secreting cells (IgSC) in the intestinal and systemic lymphoid tissues compared to non-infected pigs (4). Our hypothesis is that the lack of gut flora in Gn pigs does not cause impairment of intestinal immune responses to enteric viral infection, but the magnitude of these responses may be lower. Lactobacillus colonization may promote the development of the intestinal immune system and may have regulatory effects on the magnitude of B cell immune responses to viral infections.

OBJECTIVES
To determine the effects of lactic acid bacteria on the development of intestinal and systemic B cell responses in Gn pigs infected with RV.

MATERIALS AND METHODS

Pigs
Neonatal Gn pigs were assigned to three groups: RV-infected LAB-fed (LAB+RV+) (n=8), RV-infected non LAB-fed (LAB+RV-) (n=10), and non-infected LAB-fed (LAB+RV-) (n=4).

RV
The virulent Wa strain RV was used for oral inoculation of pigs at a dose of 1×10^6 fluorescent-forming units (FFU) at 5 days of age.

LAB acid bacteria (LAB) strains
The Lactobacilli L. reuteri strain ATCC 23272 and L. acidophilus strain NCFM™ are among the most common bacteria used in the food industry for fermentation. Pigs were orally dosed with 10^5, 10^6, 10^7 and 10^8 CFU of 1:1 mixture of L. acidophilus and L. reuteri at 3, 5, 7, 9, 11 days of age, respectively (2).

Assays
RV-specific ASC and total IgSC among mononuclear cells isolated from the Gn pigs at PID28 were enumerated using isotype-specific enzyme-linked-immunospot (ELISPOT) assays. RV-specific, peptidoglycan (PGN)-specific and total antibody titers in sera, and large and small intestinal contents (LIC and SIC) were measured using ELISAs. PGN is a cell-wall component of all gram-positive bacteria. Antibodies to PGN were measured in pigs as a proxy of antibody responses to LAB.

RESULTS

Ileum

Spleen

PBL

Figure 1. Isotype-specific IgSC responses

SUMMARY (ASC and IgSC responses - Fig. 1)

- The numbers of RV-specific IgM, IgA and IgG ASC in all lymphoid tissues of LAB+RV+ pigs were similar to those of LAB+RV+ pigs (data not shown), indicating that RV infection induced similar virus-specific B cell responses in germ-free and LAB colonized pigs.
- The numbers of total IgA SC in the ileum of LAB+RV+ pigs were significantly higher than the other groups, indicating that LAB significantly enhanced the total intestinal IgA response.
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ANTIBODY GMT

IgM IgA IgG

Figure 4. LAB colonization in Gn pigs

LAB+RV- LAB+HRV+ LAB+HRV- LAB+RV+

SUMMARY (Antibody responses - Figs. 2 and 3)

- No significant differences were observed in all isotypes of RV-specific serum and intestinal antibody titers between the LAB+RV+ and LAB+RV- pigs at any time points (data not shown).
- The serum PGN-specific IgA antibody titers in the LAB+RV+ pigs were significantly higher than LAB+RV- pigs at PID 10 (Fig 2A); the IgA and IgG antibody titers to PGN in the LIC of LAB+RV+ pigs were also higher (not significantly) than LAB+RV- pigs (data not shown), coinciding with the higher LAB counts (Fig 4).
- The serum antibody responses to PGN in the LAB+RV+ pigs were much lower (50-200 fold lower) than the RV-specific antibody responses in the LAB+RV- pigs. The PGN-specific IgM and IgG antibody titers in serum were not detectable until PID 28, indicating a weak and delayed induction of B cell responses to LAB or systemic tolerance.
- The total serum IgM and IgA antibody titers in the LAB+RV+ group were higher than those of other groups at PID 10 and 21 and the IgM were significantly higher at PID 10 (Fig 2B).
- The total IgM and IgG antibody titers in the SIC of the LAB+RV+ pigs were significantly higher than those of the LAB+RV+ pigs (Fig 3).
- The total IgM and IgG antibody titers in the LIC and IgM in the SIC of the LAB+RV+ pigs were significantly higher than those of LAB+RV- pigs (Fig 3).

SUMMARY (LAB colonization - Fig. 4)

- The two strains of LAB effectively colonized the intestinal tract of the Gn pigs, as indicated by high LAB counts in feces at PID 28, 3 weeks after the last LAB feeding.
- The LAB counts in LAB+HRV+ pigs were significantly higher than those of LAB+RV- pigs from PID 5-10.

CONCLUSIONS

- Colonization with LAB significantly enhanced the total intestinal IgA IgSC responses and total serum IgM and intestinal IgM and IgG antibody responses, but not the RV-specific ASC responses or antibody titers in Gn pigs infected with RV. Our findings demonstrated that germ-free pigs developed similar magnitude of virus-specific B cell responses to RV infection as those of the pigs colonized with commensal bacteria. However LAB colonization alone was not as efficient in promoting development of B cell compartment as RV infection in neonatal pigs.

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