Dietary Rice Bran Protects against Rotavirus Diarrhea and Promotes Th1-Type Immune Responses to Human Rotavirus Vaccine in Gnotobiotic Pigs

Xingdong Yang, Ke Wen, Christine Tin, Guohua Li, Haifeng Wang, Jacob Kocher, Kevin Pelzer, Elizabeth Ryan, Lijuan Yuan

Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA; Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA

Rice bran (RB) contains a distinct stoichiometry of phytochemicals that can promote gut mucosal immune responses against enteric pathogens. The effects of RB on rotavirus diarrhea and immunogenicity of an attenuated human rotavirus (HRV) vaccine were evaluated in gnotobiotic pigs. The four treatment groups studied were RB plus vaccine, vaccine only, RB only, and mock control. Pigs in the RB groups were fed the amount of RB that replaced 10% of the pigs’ total daily calorie intake from milk starting from 5 days of age until they were euthanized. Pigs in the vaccine groups were orally inoculated with two doses of the attenuated HRV vaccine. A subset of pigs from each group was orally challenged with the homologous virulent HRV on postinoculation day 28. Diarrhea and virus shedding were monitored daily from postchallenge day 0 to day 7. RB feeding significantly protected against diarrhea upon virulent HRV challenge and enhanced the protective rate of the vaccine against rotavirus diarrhea. Consistent with protection, RB significantly increased gamma interferon (IFN-γ)-producing CD4+ and CD8+ T cell responses in intestinal and systemic lymphoid tissues. Furthermore, RB also increased the number of total IgM- and IgA-secreting cells, total serum IgM, IgG, and IgA titers, and HRV-specific IgA titers in intestinal contents. RB reduced the numbers of intestinal and systemic HRV-specific IgA and IgG antibody-secreting cells and reduced serum HRV-specific IgA and IgG antibody titers before the challenge. These results demonstrate clear beneficial effects of RB in protection against rotavirus diarrhea and stimulation of nonspecific and HRV-specific immune responses, as well as its biased Th1-type adjuvant effect for the vaccine.

Rice bran (RB), a globally accessible, abundant, and underutilized agricultural by-product, has a distinct stoichiometry of bioactive compounds, phytochemicals, and minerals (1). It has been studied for bioactive functions such as the prevention and treatment of chronic diseases, growth of beneficial intestinal microbes, induction of mucosal and systemic immune responses, and protection against enteric pathogens (2–5). Thus, this agricultural by-product represents a promising and practical diet-based solution for increasing the innate resistance against enteric pathogens that cause diarrhea. In particular, because of its immune stimulatory functions, it can be potentially used as a vaccine adjuvant for enteric pathogen infections. Given that RB can support colonization of gut probiotics (e.g., Lactobacillus spp.), enhance mucosal IgA production (2), and significantly reduce the enteric burden of Salmonella infection in mice (5), continued investigation of the mechanisms of dietary RB in its protection against viral pathogens that cause significant global morbidity and mortality (e.g., rotavirus) is warranted.

Previous studies have shown the immunomodulatory effects of RB on both innate and adaptive immunity in vitro and in vivo (4). RB oil enhanced T and B lymphocyte proliferation, production of Th1 cytokines (interleukin 2 [IL-2], gamma interferon [IFN-γ], and tumor necrosis factor alpha [TNF-α]) by lymphocytes, and reduced Th2 cytokines (both serum- and lymphocyte-derived IL-4), as well as the level of serum IgE and IgG1 (3). MGN-3, an arabinoxylan derived from rice bran, also increased the levels of Th1 cytokines in human multiple myeloma patients (6). Importantly, γ-oryzanol significantly promoted the development of antibody responses in rats stimulated with sheep red blood cells (4). Total local (feces) and systemic (serum) IgA levels and IgA expression of Peyer’s patches B cells were also enhanced in mice fed a 10% RB diet, suggesting that RB promoted both mucosal and systemic B cell development (2). These studies demonstrated the immunostimulatory effects of RB on multiple components of the immune system.

Given the RB-mediated protection against bacterial pathogens (7–9) and the stimulatory effects on both the innate and adaptive immune systems, RB represents a promising natural food product for modulating mucosal immunity and protecting against diarrhea from major enteric pathogens such as human rotavirus. The gnotobiotic (Gn) pig model of human rotavirus (HRV) infection and diarrhea has been extensively utilized to study HRV infection and vaccination (10–14). In this study, using the well-established neonatal Gn pig model, we aimed to (i) determine whether RB can reduce the susceptibility to infection and diarrhea upon virulent HRV challenge and (ii) examine the ability of RB to promote the development of intestinal and systemic T and B cell immune re-
sponses and improve the protective efficacy of oral rotavirus vaccine compared to that of the control diet.

**MATERIALS AND METHODS**

*Gn pig experimental groups and treatment.* Neonatal Gn pigs were derived and maintained in sterile isolators as previously described (15). The four treatment groups studied were RB plus attenuated HRV (AttHRV) vaccine, AttHRV vaccine only, RB only, and mock control. Heat-stabilized, gamma-irradiated RB (from the Neptune variety), produced from the 35th passage of the virus strain (GIP1A[8]) HRV in the MA104 cell culture (16). Two oral doses of the AttHRV vaccine were given at approximately 5 × 10^7 focus-forming units (FFU)/dose on PPD 5 and PPD 15. The mock group received neither RB nor AttHRV vaccine. A subset of pigs from each group was orally challenged with the virulent HRV (VirHRV) Wa strain (GIP1A[8]) at a dose of approximately 1 × 10^7 FFU on post-first inoculation day (PID) 28. The 50% infectious dose (ID_{50}) of the virulent HRV in neonatal Gn pigs was determined to be approximately 1 FFU (17). To determine the ID_{50} for pigs of the virulent HRV strain, starting at 5 days of age (PPD 5) until the end of the experiment. The amounts of RB were calculated to replace 10% of the pigs’ daily calorie intake from milk. AttHRV is produced from the 35th passage of Wa strain (GIP1A[8]) HRV in the MA104 cell culture (16). Two oral doses of the AttHRV vaccine were given at approximately 5 × 10^7 focus-forming units (FFU)/dose on PPD 5 and PPD 15. The mock group received neither RB nor AttHRV vaccine. A subset of pigs from each group was orally challenged with the virulent HRV (VirHRV) Wa strain (GIP1A[8]) at a dose of approximately 1 × 10^7 FFU on post-first inoculation day (PID) 28. The 50% infectious dose (ID_{50}) of the virulent HRV in neonatal Gn pigs was determined to be approximately 1 FFU (17). To measure antibody titers, blood samples were collected weekly starting at PPD 5 and upon euthanization. Rectal swabs were taken daily to monitor virus shedding and diarrhea from postchallenge day (PCD) 0 to PCD 7. Pigs were euthanized on PID 28 or PCD 7, and mononuclear cells (MNCs) from ileum, spleen, and blood were isolated for detection of T and B cell responses using flow cytometry and enzyme-linked immunosorbent assay (ELISPOT) analysis. All animal experiments were conducted according to the protocols approved by the institutional animal care and use committee at the Virginia Polytechnic Institute and State University.

**Detection of virus shedding by ELISA.** Rectal swabs taken daily upon VirHRV challenge were processed by washing two swabs in 8 ml diluent 5 (minimal essential medium [MEM], 1% penicillin and streptomycin, 1% HEPES) and then centrifuged at 2,100 rpm for 15 min at 4°C. supernatants were then divided into aliquots and stored at −20°C. Virus antigens in the fecal swabs were detected by enzyme-linked immunosorbent assay (ELISA) (18). Rectal swabs from mock treatment Gn pigs were used as negative controls.

**Detection of total CD3^+ CD4^+ (Th) cells, CD3^+ CD8^+ (cytotoxic T lymphocyte [CTL]) cells, and IFN-γ-producing T cell responses by flow cytometry.** The frequencies of total and IFN-γ-producing CD4^+ and CD8^+ T cells among CD3^+ mononuclear cells in ileum, intraepithelial lymphocytes (IEL), spleen, and blood were determined by intracellular staining and flow cytometry according to a previous publication (19). Data were acquired using a BD FACSAria flow cytometer, and data were analyzed using FlowJo 7.22 (Tree Star, Inc.).

Detection of total immunoglobulin-secreting cells, HRV-specific antibody-secreting cells, total and HRV-specific serum, and intestinal antibody titers. The ELISPOT assays for rotavirus-specific antibody-secreting cell (ASC) response and data reporting were performed as previously described (16, 20, 21). The ELISPOT assay for measuring the total immunoglobulin-secreting cell (IgSC) response and data reporting followed a previously described protocol (16). To determine the HRV-specific IgA and IgG antibody titers in serum and intestinal contents, isotype-specific ELISA was performed according to the protocol previously described (22, 23). Total IgM, IgA, and IgG antibody titers in serum and intestinal contents were assessed by following an established ELISA protocol (24).

**Statistical analysis.** The Kruskal-Wallis rank sum test was used to compare data on virus shedding and diarrhea, Th and CTL cells, IFN-γ, CD4^+ and CD8^+ T lymphocytes, total IgSC antibody-secreting cells, and HRV-specific ASCs. Fisher’s exact test was used to compare the percentages of virus shedding and diarrhea. Total and HRV-specific antibody titers in the serum were analyzed using the analysis of variance (ANOVA) general linear model (GLM). Total and HRV-specific antibody titers in the large and small intestinal contents were analyzed using ANOVA and Tukey’s test.

**RESULTS**

**RB reduced HRV diarrhea but not virus shedding.** The effects of RB on rotavirus infection and disease were determined by comparing the virus shedding and diarrhea parameters among the four treatment groups, i.e., RB-AttHRV, AttHRV only, RB only, and mock control. The results summarized in Table 1 showed that compared to the mock control diet group, the RB-only group had a significantly lower incidence (100% versus 20%), shorter mean duration (5.6 versus 0.2 days), and reduced severity of diarrhea (diarrhea score, 14.4 versus 4.4). Compared to the AttHRV group, RB-AttHRV vaccine-treated Gn pigs had a significantly reduced incidence of diarrhea (67% versus 0%), shorter mean duration (4.6 versus 0 days), and reduced severity of diarrhea (diarrhea score, 9.8 versus 4.4). Importantly, the RB-only group had less diarrhea than the group with AttHRV vaccine alone, with a reduced incidence (20% versus 67%, respectively) of diarrhea, significantly shorter mean duration (0.2 versus 4.6 days, respectively), and lower diarrhea scores (4.4 versus 9.8, respectively).

**TABLE 1 Clinical signs and rotavirus fecal shedding in Gn pigs after VirHRV challenge**

| Treatment          | n  | % with diarrhea | Mean days to onset | Mean duration (days) | Mean cumulative score | % shedding virus | Mean days to onset | Mean duration (days) |
|--------------------|----|-----------------|--------------------|----------------------|-----------------------|-----------------|--------------------|----------------------|
| RB plus AttHRV     | 6  | 0 C             | 8 (0) C            | 0 (0) C              | 4.4 (1.2) C           | 100 A,B         | 2.0 (0.5) B       | 3.2 (0.91) B         |
| AttHRV only        | 12 | 67 A,B          | 4.8 (0.8) B        | 4.6 (0.5) B          | 9.8 (1.4) B           | 50 B            | 6.0 (0.7) A       | 1.3 (0.2) C          |
| RB only            | 5  | 20 B,C          | 7.2 (0.8) A,B      | 0.2 (0.2) C          | 4.4 (1.6) C           | 100 A,B         | 1.6 (0.2) B       | 6.2 (0.2) A          |
| Mock               | 9  | 100 A           | 1.4 (0.2) C        | 5.6 (0.3) A          | 14.4 (1.0) A          | 100 A           | 2.0 (0.3) B       | 4.7 (0.7) A,B       |

\(a\) Pigs with daily fecal scores of 1–2 were considered diarrheic. Fecal consistency was scored as 0 (normal), 1 (pasty), 2 (semiliquid), or 3 (liquid).

\(b\) Mean cumulative score calculation included all the pigs in each group.

\(c\) Numbers in parentheses are standard errors of the mean. In the groups in which some but not all pigs had diarrhea or shedding, the onset of diarrhea or shedding for nondiarrheic/shed pigs was designated day 8 for calculating the mean days to onset.

\(d\) For days of diarrhea and virus shedding, if there was no diarrhea or virus shedding until the euthanasia day (PCD 7), the duration was recorded as 0 days.

\(e\) Fisher’s exact test was used for comparisons. Different letters (A, B, and C) indicate significant differences in protection rates among groups (\(P < 0.05\)), while shared letters indicate no significant difference.

\(f\) Kruskal-Wallis rank sum test was used for comparisons. Different letters (A, B, and C) indicate significant differences in protection rates among groups (\(P < 0.05\)), while shared letters indicate no significant difference.

October 2014 Volume 21 Number 10 cvlasm.org 1397

Downloaded from http://cvi.asm.org on July 20, 2018 by guest

Effect of Rice Bran on Human Rotavirus Immunity
No significant difference in virus shedding was observed between the RB-only group and mock controls. Compared to the AttHRV vaccine group, the RB-AttHRV group had increased virus shedding (50% versus 100%, respectively), a significantly earlier onset (6.0 versus 2.0 days, respectively), and a significantly longer mean duration of virus shedding (1.3 versus 3.2 days, respectively). In addition, the RB-only group had a slightly longer mean duration of virus shedding (6.2 versus 4.7 days; not significant) than the mock controls. These data suggest that RB protects against rotavirus-induced diarrhea through mechanisms that are independent of affecting rotavirus replication.

Effect of RB on total Th and CTL development in intestinal and systemic lymphoid tissues. The frequency of total Th cells and CTL among lymphocytes in different tissues on PID 28 were determined. The results are shown in Fig. 1. Compared to the mock control group, the RB-only group had similar frequencies of total Th and CTL T cells among lymphocytes in both intestinal (ileum and IEL) and systemic (spleen and blood) lymphoid tissues. Similarly, there were no significant differences between the AttHRV group and the RB-AttHRV group, except for a significantly downregulated CTL response in the IEL of the RB-AttHRV group. These results suggest that RB did not influence the development of total Th and CTL cells.

Rice bran enhanced IFN-γ+ CD4+ and CD8+ T cell responses. Effector T cell responses against rotavirus are an important protective mechanism against infection. The effects of RB on effector T cells were assessed by the frequency of IFN-γ-producing CD4+ and CD8+ T cell populations among total CD3+ mononuclear cells in both intestinal tissues (ileum and IEL) and systemic lymphoid tissues (spleen and blood). The results are shown in Fig. 2. Compared to the mock control group, the RB-only group had significantly increased frequencies of IFN-γ+ CD4+ T cell populations in ileum, spleen, and blood on PID 28 and IFN-γ+ CD8+ T cell populations in ileum, spleen, and blood on PID 28 and PCD 7. Compared to the AttHRV vaccine group, the RB-AttHRV group had significantly increased frequencies of IFN-γ+ CD4+...
and IFN-γ+ CD8+ T cell populations in the ileum, spleen, and blood on PID 28 and PCD 7, except for IFN-γ+ CD4+ T cells in the ileum and spleen on PCD 7. There were no significant differences in IEL at any time point. These data demonstrate that RB has strong stimulating effects that favor Th1-type immune responses.

**Rice bran promoted the development of intestinal and systemic IgSCs.** Total immunoglobulins in the intestinal and systemic tissues, particularly intestinal IgA, play a significant role in nonspecific mucosal protection against viral infections. The numbers of IgM, IgA, and IgG IgSCs in the ileum, spleen, and blood were measured by ELISPOT assay and compared between the RB-only group and the mock group (Fig. 3). The RB-only group showed significantly increased numbers of IgM IgSCs in ileum and spleen and in the numbers of IgA IgSCs in spleen and blood at PID 28. The numbers of IgA IgSCs in the ileum and the numbers of IgG IgSCs in all tissues did not differ significantly between the RB-only and mock control groups. These data indicated that dietary RB intake can promote the development of intestinal and systemic IgSCs (IgM in ileum and spleen, IgA in spleen and blood).

**Rice bran stimulated the production of total IgM, IgA, and IgG in serum.** Total serum IgM, IgA, and IgG antibody titers in Gn pigs fed with or without RB were determined using ELISA, and the results are shown in Fig. 4. On PID 21, RB-only pigs had significantly higher IgM and IgA titers than in the controls. Additionally, RB-only pigs had significantly higher serum IgM, IgA, and IgG antibody titers than the controls on PID 28. After the HRV challenge, RB-only pigs had significantly higher IgA titers than the controls. In addition, RB-AttHRV pigs had significantly higher IgM and IgA titers on PID 28 and of IgM titers on PCD 7 than the AttHRV-only pigs. These data demonstrated that RB promoted the production of total serum IgM, IgA, and IgG antibody titers in both naive and vaccinated Gn pigs.

**FIG 2** IFN-γ-producing CD4+ and CD8+ T cell responses in control or vaccinated Gn pigs fed with or without RB-supplemented diet. MNCs from Gn pigs in each treatment group euthanized on PID 28 (left panel) or PCD 7 (right panel) were analyzed by flow cytometry after the MNCs were stimulated with semipurified AttHRV antigen for 17 h. The frequencies of IFN-γ-producing T cells among total CD3+ cells from each tissue were represented by frequencies of the IFN-γ+ CD4+ (top panel) or IFN-γ+ CD8+ (bottom panel) T cell subsets among CD3+ cells in the respective tissues shown on the x axis. Error bars indicate standard errors of the means. Different capital letters (A, B, and C) indicate significant differences between groups (P < 0.05), while shared letters indicate no significant difference (Kruskal-Wallis rank sum test, P < 0.05; n = 3 to 6 for PID 28 and n = 4 to 12 for PCD 7).

**FIG 3** Mean numbers of total IgSCs in Gn pigs fed with or without RB-supplemented diet. MNCs from Gn pigs in RB-only and mock groups euthanized on PID 28 (without AttHRV vaccine and HRV challenge) were analyzed by total IgSC ELISPOT assay. Numbers on the y axis indicate the number of total IgM-, IgA-, or IgG-secreting cells per $5 \times 10^5$ MNCs in the respective tissues shown on the x axis. Error bars indicate standard errors of the means. Different capital letters (A and B) indicate significant differences between groups (P < 0.05), while shared letters indicate no significant difference (Kruskal-Wallis rank sum test, P < 0.05; n = 3 to 4).
Rice bran decreased the intestinal and systemic HRV-specific IgA and IgG ASC responses to AttHRV vaccination but not VirHRV challenge. HRV-specific serum IgA levels and the numbers of intestinal IgA and IgG ASCs have been associated with protection against rotavirus infection and diarrhea (16, 23). HRV-specific ASC responses are shown in Fig. 5. Compared to the AttHRV-alone group, the RB-AttHRV group had significantly lower numbers of both IgA and IgG ASCs in the ileum, spleen, and blood on PID 28. On PCD 7, compared to the nonvaccinated RB-only and mock groups, both the AttHRV-only and the RB-AttHRV groups had significantly higher numbers of HRV-specific IgA and IgG ASCs in the ileum. The two vaccinated groups also had significantly higher numbers of IgA ASCs in the blood than the mock group. The RB-only group had significantly higher numbers of IgA ASCs in the ileum and blood than those in the mock group. Together, these results demonstrated that RB downregulated virus-specific IgA and IgG effector responses.

![Graph showing total serum IgM, IgA, and IgG antibody responses in control or vaccinated Gn pigs fed with or without RB-supplemented diet.](http://cvi.asm.org/)

![Graph showing mean numbers of HRV-specific ASCs in Gn pigs fed with or without RB-supplemented diet.](http://cvi.asm.org/)
responses induced by the AttHRV vaccine at PID 28 but not memory B cell responses upon VirHRV challenge.

Rice bran reduced serum HRV-specific IgA and IgG antibody responses to AttHRV. To further confirm the results indicating that RB downregulated HRV-specific IgA and IgG ASC responses at PID 28, HRV-specific serum IgA and IgG antibody titers were determined by ELISA (Fig. 6). Consistent with HRV-specific IgA ASC data, serum IgA titers were significantly lower on both PID 21 and PID 28, but with no significant difference on PCD 7, in the RB-AttHRV group than in the AttHRV vaccine group. For both the RB-AttHRV and AttHRV-only groups, HRV-specific serum IgG antibody titers were not significantly different on PID 21, PID 28, and PCD 7, although the RB-AttHRV group had significantly higher IgG antibody titers on PID 10. The RB-only group had significantly lower virus-specific IgG antibody titers than the mock group on PCD 7.

Rice bran increased HRV-specific IgA titers in the intestinal contents. Total immunoglobulins and HRV-specific antibody responses in the small intestinal contents (SIC) and large intestinal contents (LIC) were measured by ELISAs (Fig. 7). RB did not significantly change the levels of total immunoglobulins (IgA, IgG, and IgM) in the intestinal contents on PID 28 or PCD 7, except for the decreased total IgA titer in LIC on PID 28 compared to that in control pigs on PID 28 (Fig. 7A). It is important to note that HRV-specific IgA titers in both the SIC and LIC of the RB-AttHRV pigs were higher at PID 28 and significantly higher at PCD 7 than in those in the AttHRV-only pigs (Fig. 7B). These data demonstrated that RB can enhance the production of virus-specific IgA antibodies by intestinal memory B cells in the AttHRV-vaccinated pigs after VirHRV challenge, even though the numbers of virus-specific IgA ASCs (Fig. 5) and the titers of virus-specific IgA antibodies in serum before challenge were reduced (Fig. 6).

DISCUSSION
In this study, we examined the effects of RB supplementation on rotavirus infection and diarrhea, the total and virus-specific T and B cell responses, and isotype-specific antibody responses induced

FIG 6 HRV-specific IgA and IgG antibody titers in serum of Gn pigs fed with or without RB-supplemented diet. Antibody titers were measured by ELISA and are presented as geometric mean titers for each treatment group. Error bars indicate standard errors of the means. Different capital letters (A, B, and C) indicate significant differences among different treatment groups for the same time point, while different lowercase letters (a, b, c, d, and e) indicate significant differences among different time points for the same treatment group. Shared capital or lowercase letters indicate no significant difference (ANOVA, general linear model [GLM], \( P < 0.05; n = 10 \) to 18).

FIG 7 Total and HRV-specific IgA and IgG antibody titers in small intestinal contents (SIC) and large intestinal contents (LIC) of Gn pigs fed with or without RB-supplemented diet. Antibody titers in intestinal contents were measured by ELISA and are presented as geometric mean titers for each treatment group. Error bars indicate standard errors of the means. Different capital letters (A and B) indicate significant differences among treatment groups for the same time point, while shared letters indicate no significant difference (ANOVA, Tukey’s test, \( P < 0.05; n = 3 \) to 6 for PID 28 and \( n = 4 \) to 12 for PCD 7).
by the AttHRV vaccine using neonatal Gn pigs as a model system. We observed that 10% dietary RB supplementation to milk significantly protected against rotavirus diarrhea but did not reduce rotavirus replication. RB also strongly promoted the development of IFN-γ-producing T cells, IgM- and IgA-producing IgSCs, total serum IgM, IgA, and IgG antibodies, and HRV-specific intestinal IgA production but significantly reduced HRV-specific IgA and IgG ASCs in intestinal and systemic lymphoid tissues and HRV-specific serum IgA production at PID 28.

Rice bran alone reduced rotavirus diarrhea incidence and severity without reducing rotavirus shedding. Surprisingly, while RB and AttHRV vaccine synergistically and completely protected against rotavirus diarrhea, the RB reduced the protection of AttHRV vaccine against rotavirus shedding. These results strongly suggest that mechanisms by which RB protects against rotavirus diarrhea are independent of rotavirus infection. The underlying mechanisms for rotavirus-induced diarrhea are not completely understood. The pathogenesis of rotavirus-induced diarrhea has been reviewed (25, 26). Four distinct but nonexclusive mechanisms have been implicated, including (i) malabsorption due to the destruction of absorptive enterocytes in the villus, caused by rotavirus infection and increased intracellular [Ca2+]i, (ii) NSP4 enterotoxin-mediated increases in membrane permeability and tight junction disruption, (iii) increased secretion from the crypt cells and intestinal motility via stimulation of the enteric nervous system by rotavirus or NSP4 enterotoxin, and (iv) villus ischemia caused by unidentified vasoactive substances during rotavirus infection. RB could interfere with each of these four mechanisms. In fact, extracts from RB have been shown to be effective in reducing diarrhea through inhibition of the intestinal mucosal Cl− ion secretion by intestinal epithelial cells (27, 28). This mechanism is likely to have contributed to the protective effects of RB against rotavirus-induced diarrhea in the current study. It was also reported that zinc and enkephalinase inhibitors attenuate rotavirus-induced diarrhea (26). Certain RB phytochemicals might have functioned as such inhibitors. Further studies are under way to examine the effects of RB on the intestinal barrier integrity and permeability during rotavirus infection.

Both effector T and B cell responses play important roles during rotavirus infection and are associated with the protective efficacy of rotavirus vaccine against rotavirus infection and diarrhea (19, 23, 29). The significantly increased frequencies of IFN-γ-producing CD4+ and CD8+ T cell responses in local (ileum) and systemic (spleen and blood) lymphoid tissues at both PID 28 and PCD 7 suggest that RB promoted the development of effector T cell responses. However, this effect was not due to the enhanced expansion of total Th and CTL cells, as RB did not significantly increase their frequencies among lymphocytes in both intestinal and lymphoid tissues.

Rice bran also significantly enhanced the development of total IgM IgSCs in ileum and spleen and IgA IgSCs in spleen and blood and levels of total serum IgM, IgA, and IgG antibodies pre- and postchallenge, as well as rotavirus-specific IgA antibody levels in intestinal contents after challenge, indicating the stimulatory effect of RB on the development of total and specific B cell responses to the AttHRV vaccine and VirHRV challenge. Similarly, a previous study showed that the number of peripheral blood lymphocytes was significantly increased in Wistar male rats fed a diet with 10% hemicellulose extracted from RB fiber (RBF) for 2 weeks (30). However, the significantly reduced numbers of rotavirus-specific IgA and IgG ASCs in both local and systemic tissues and the correspondingly lower rotavirus-specific serum IgA and IgG antibody titers on PID 28 suggest that the immunostimulatory effects (adjuvanticity) of RB are biased toward Th1 T cell responses before challenge and are antigen specific. Thus, RB functioned as a Th1-type immune response “food adjuvant” for the AttHRV vaccine. This observation is consistent with those of previous studies showing that RB feeding in mice upregulated Th1 cytokines and downregulated Th2 and antibody responses. The reduction in rotavirus-specific B cell and serum antibody response at challenge may have contributed to the increased fecal rotavirus shedding in the RB-AttHRV treatment group over the AttHRV-only treatment group. However, RB did not negatively affect the rotavirus-specific memory B cell responses and enhanced rotavirus-specific intestinal IgA antibody responses at PCD 7, suggesting that RB increased priming of local virus-specific B cells even under the Th1-biased condition before challenge. The molecular mechanisms and kinetics by which RB modulates T and B cell responses warrant further study.

In summary, results from the current study demonstrate that RB significantly reduced the susceptibility to rotavirus diarrhea without reducing rotavirus shedding upon virulent HRV challenge in Gn pigs compared to the control diet. Furthermore, RB promoted the development of intestinal and systemic IFN-γ-producing CD4+ and CD8+ T cell responses, total IgM IgSCs in ileum and spleen, total IgA IgSCs in spleen and blood, and total serum IgM, IgA, and IgG antibody production. Additionally, RB increased HRV-specific IgA titers in the intestinal contents postchallenge. RB alone also significantly increased the virus-specific IgA ASC response postchallenge in ileum and blood. These results have significant clinical implications for the prevention and management of enteric pathogen-induced diarrhea using dietary RB in developing countries. Clinical trials should be conducted before RB is recommended for use alone and in combination with rotavirus and other vaccines to reduce diarrheal diseases and to improve human health.

ACKNOWLEDGMENTS

This work was supported by the Bill and Melinda Gates Foundation Grand Challenge Exploration in Global Health grant OPP1043255 to E.R., with a G-6289 subcontract to L.Y., and a grant (R01AT004789) from the National Center of Complementary and Alternative Medicine, National Institutes of Health, Bethesda, MD, to L.Y.

We thank Marlice Vonck, Sherrie Clark-Deener, Pete Jobst, Andrea Pulliam, Kim Allen, and Shannon Viers for animal care. We thank Mariah Weiss for assistance in animal care and experiments and Melissa Makris for assistance in flow cytometry. The AttHRV and VirHRV strains were from Linda J. Saif, The Ohio State University.

REFERENCES

1. Ryan EP. 2011. Bioactive food components and health properties of rice bran. J. Am. Vet. Med. Assoc. 238:593–600. http://dx.doi.org/10.2460/javma.238.5.593.
2. Henderson AJ, Kumar A, Barnett B, Dow SW, Ryan EP. 2012. Consumption of rice bran increases mucosal immunoglobulin A concentrations and numbers of intestinal Lactobacillus spp. J. Med. Food 15:469–475. http://dx.doi.org/10.1089/jmf.2011.0213.
3. Sierra S, Lara-Villoslada F, Olivares M, Jimenez J, Boza J, Xaus J. 2005. Increased immune response in mice consuming rice bran oil. Eur. J. Nutr. 44:509–516. http://dx.doi.org/10.1007/s00394-005-0554-y.
4. Ghatak SB, Panchal SJ. 2012. Investigation of the immunomodulatory potential of oryzanol isolated from crude rice bran oil in experimental
animal models. Phytother. Res. 26:1701–1708. http://dx.doi.org/10.1002/ptr.4627.
5. Kumar A, Henderson A, Forster GM, Goodyear AW, Weir TL, Leach JE, Dow SW, Ryan EP. 2012. Dietary rice bran promotes resistance to Salmonella enterica serovar Typhimurium colonization in mice. BMC Microbiol. 12:71. http://dx.doi.org/10.1186/1471-2180-12-71.
6. Cholujova D, Jakubikova I, Czako B, Martisova M, Hunakova L, Duraj J, Mistrick M, Sedlak I. 2013. MGN-3 arabinoxylan rice bran modulates innate immunity in multiple myeloma patients. Cancer Immunol. Immunother. 62:437–445. http://dx.doi.org/10.1007/s00262-012-1344-z.
7. Ray B, Hutterer C, Bandypodhuy SS, Ghosh K, Chatterjee UR, Ray S, Zeittrager I, Wagner S, Marschall M. 2013. Chemically engineered sulfated glucans from rice bran exert strong antiviral activity at the stage of viral entry. J. Nat. Prod. 76:2180–2188. http://dx.doi.org/10.1021/np4003977.
8. Ghosh T, Auerochs S, Saha S, Ray B, Marschall M. 2010. Anticytomegalovirus activity of sulfated glucans generated from a commercial preparation of rice bran. Antivir. Chem. Chemother. 21:85–95. http://dx.doi.org/10.3851/IMP1685.
9. Ghoneum R, Kumar M. 1998. Anti-HIV activity in vitro of MGN-3, an activated arabinoxylan from rice bran. Biochem. Biophys. Res. Commun. 243:25–29. http://dx.doi.org/10.1006/bbrc.1997.8047.
10. Liu F, Li G, Wen K, Wu S, Zhang Y, Bui T, Yang X, Kocher J, Sun J, Jortner B, Yuan L. 2013. Lactobacillus rhamnosus GG on rotavirus-induced injury of ileal epithium in gnotobiotic pigs. J. Pediatr. Gastroenterol. Nutr. 57:750–758. http://dx.doi.org/10.1097/MGP.0b013e3182a356e1.
11. Liu F, Wen K, Li G, Yang X, Kocher J, Bui T, Jones D, Pelzer K, Clark-Deener S, Yuan L. 2014. Dual functions of Lactobacillus acidophilus NCFM at the intermediate dose in protection against rotavirus diarrhea in gnotobiotic pigs vaccinated with a human rotavirus vaccine. J. Pediatr. Gastroenterol. Nutr. 58:171–178. http://dx.doi.org/10.1097/MGP.0000000000000197.
12. Wen K, Li G, Bui T, Liu F, Li Y, Kocher J, Lin L, Yang X, Yuan L. 2012. High dose and low dose Lactobacillus acidophilus exerted differential immune modulating effects on T cell immune responses induced by an oral human rotavirus vaccine in gnotobiotic pigs. Vaccine 30:1198–1207. http://dx.doi.org/10.1016/j.vaccine.2011.11.107.
13. Wen K, Li G, Zhang W, Azevedo MS, Saif LJ, Liu F, Bui T, Yousef A, Yuan L. 2011. Development of gammapelta T cell subset responses in gnotobiotic pigs infected with human rotaviruses and colonized with probiotic lactobacilli. Vet. Immunol. Immunopathol. 141:267–275. http://dx.doi.org/10.1016/j.vetimm.2010.04.016.
14. Yuan L, Saif LJ. 2002. Induction of mucosal immune responses and protection against enteric viruses: rotavirus infection of gnotobiotic pigs as a model. Vet. Immunol. Immunopathol. 87:147–160. http://dx.doi.org/10.1016/S0165-2427(02)00046-6.
15. Meyer RC, Bohl EH, Kohler EM. 1964. Procurement and maintenance of germ-free swine for microbiological investigations. Appl. Microbiol. 12:295–300.
16. Yuan L, Ward LA, Rosen BI, To TL, Saif LJ. 1996. Systemic and intestinal antibody-secreting cell responses and correlates of protective immunity to human rotavirus in a gnotobiotic pig model of disease. J. Virol. 70:3073–3083.
17. Ward LA, Rosen BI, Yuan L, Saif LJ. 1996. Pathogenesis of an attenuated and a virulent strain of group A human rotavirus in neonatal gnotobiotic pigs. J. Gen. Virol. 77:1431–1441. http://dx.doi.org/10.1099/0022-1317-77-7-1431.
18. Liu F, Li G, Wen K, Bui T, Cao D, Zhang Y, Yuan L. 2010. Porcine small intestinal epithelial cell line (IPEC-J2) of rotavirus infection as a new model for the study of innate immune responses to rotaviruses and protection. Viral Immunol. 23:135–149. http://dx.doi.org/10.1089/vim.2009.0088.
19. Yuan L, Wen K, Azevedo MS, Gonzalez AM, Zhang W, Saif LJ. 2008. Virus-specific intestinal IFN-gamma-producing T cell responses induced by human rotavirus infection and vaccines are correlated with protection against rotavirus diarrhea in gnotobiotic pigs. Vaccine 26:3322–3331. http://dx.doi.org/10.1016/j.vaccine.2008.03.085.
20. Yuan L, Kang SY, Ward LA, To TL, Saif LJ. 1998. Antibody-secreting cell responses and protective immunity assessed in gnotobiotic pigs inoculated orally or intramuscularly with inactivated human rotavirus. J. Virol. 72:330–338.
21. Yuan L, Azevedo MS, Gonzalez AM, Jeong KI, Van Nguyen T, Lewis P, Iosef C, Herrmann JE, Saif LJ. 2005. Mucosal and systemic antibody responses and protection induced by a prime/boost rotavirus-DNA vaccine in a gnotobiotic pig model. Vaccine 23:3925–3936. http://dx.doi.org/10.1016/j.vaccine.2005.03.009.
22. Parreno V, Hodgins DC, de Arriba L, Kang SY, Yuan L, Ward LA, To TL, Saif LJ. 1999. Serum and intestinal isotype antibody responses to Wa human rotavirus in gnotobiotic pigs are modulated by maternal antibodies. J. Gen. Virol. 80:1417–1428.
23. To TL, Ward LA, Yuan L, Saif LJ. 1998. Serum and intestinal isotype antibody responses and correlates of protective immunity to human rotavirus in a gnotobiotic pig model of disease. J. Gen. Virol. 79:2661–2672.
24. Zhang W, Azevedo MS, Gonzalez AM, Saif LJ, Van Nguyen T, Wen K, Yousef AE, Yuan L. 2008. Influence of probiotic Lactobacillus colonization on neonatal B cell responses in a gnotobiotic pig model of human rotavirus infection and disease. Vet. Immunol. Immunopathol. 122:175–181. http://dx.doi.org/10.1016/j.vetimm.2007.10.003.
25. Ramig RF. 2004. Pathogenesis of intestinal and systemic rotavirus infection. J. Virol. 78:10213–10220. http://dx.doi.org/10.1128/JVI.78.19.10213-10220.2004.
26. Hagbom M, Sharma S, Lundgren O, Svansson L. 2012. Towards a human rotavirus disease model. Curr. Opin. Virol. 2:408–418. http://dx.doi.org/10.1016/j.coviro.2012.05.006.
27. Goldberg ED, Saltzman JR. 1996. Rice inhibits intestinal secretions. Nutr. Rev. 54:36–37.
28. Mathews CJ, MacLeod RJ, Zheng SX, Hanrahan JW, Bennett HP, Hamilton JR. 1999. Characterization of the inhibitory effect of boiled rice on intestinal chloride secretion in guinea pig crypt cells. Gastroenterology 116:1342–1347. http://dx.doi.org/10.1016/S0016-5085(99)70498-1.
29. Blutt SE, Miller AD, Salmon SL, Metzger DW, Conner ME. 2012. IgA is important for clearance and critical for protection from rotavirus infection. Mucosal Immunol. 5:712–719. http://dx.doi.org/10.1038/mi.2012.15.
30. Takenaka S, Itoyama Y. 1993. Rice bran hemorrholce increases the peripheral blood lymphocytes in rats. Life Sci. 52:9–12. http://dx.doi.org/10.1016/0022-3205(93)90282-8.