Ascaris suum (A. suum) can alter local immune responses and improve intestinal function during a nematode infection by reducing components of a strong allergenic type-2 response in the pig without compromising normal parasitic expulsion.

**ABSTRACT**

An evaluation of a localized intestinal allergic type-2 response concomitant with consumption of probiotic bacteria is not well documented. This study investigated the effect of feeding probiotic *Bifidobacterium animalis subspecies lactis* (Bb12) or a placebo in weaned pigs that were also inoculated with *Ascaris suum* (A. suum) eggs to induce a strong Th2-dependent allergic type 2 immune response. Sections of jejunal mucosa were mounted in Ussing chambers to determine changes in permeability and glucose absorption, intestine and liver samples were collected for analysis of type-2 related gene expression, jejenum examined histologically, and sera and intestinal fluid were assayed for parasite antigen specific antibody. The prototypical parasite-induced secretory response to histamine and reduced absorption of glucose in the jejenum were attenuated by feeding Bb12 without a change in mucosal resistance. Parasite antigen-specific IgA response in the serum and IgG1 and IgG2 response in the ileal fluid were significantly increased in *A. suum*-infected pigs treated with Bb12 compared to infected pigs given the placebo. *Ascaris suum*-induced eosinophilia in the small intestinal mucosa was inhibited by Bb12 treatment without affecting the normal expulsion of *A. suum* 4th stage larvae (L4) or the morphometry of the intestine. Expression of genes associated with Th1/Th2 cells, Treg cells, mast cells, and physiological function in the intestine were modulated in *A. suum* infected-pigs treated with Bb12. These results suggested that Bb12 can alter local immune responses and improve intestinal function during a nematode infection by reducing components of a strong allergenic type-2 response in the pig without compromising normal parasitic expulsion.

**Introduction**

Two major functions of the intestinal epithelium are to provide a selective barrier to luminal contents and to transport water, nutrients, and electrolytes. The intestinal epithelium is also the interface between luminal contents and the mucosal immune system. It functions not only to reduce responses to food antigens and commensal bacteria, but to activate host defenses against pathogens. Alterations in epithelial barrier function, therefore, can play a significant role in regulating gastrointestinal disease.

Infection with gastrointestinal parasites affects the physiological and immunological responses of the host. Helminth infection induces a hyper-contractility of intestinal smooth muscle, inhibition of intestinal glucose absorption, and changes in intestinal secretion that are IL-4 receptor-dependent and STAT6-inducible. There is growing interest in the use of probiotics to ameliorate the negative effects of infection and allergic disease. Probiotics have been used to treat a variety of gastrointestinal (GI) ailments including inflammatory bowel disease (IBD), irritable bowel syndrome, viral enteritis, and antibiotic-associated diarrhea. Although little is known definitively about their mechanism of action, probiotics seem to have protective and anti-inflammatory effects on the intestinal mucosa. Proposed mechanisms include the production of ammonia, hydrogen peroxide, and bacteriocins that inhibit the growth of pathogenic bacteria,
competition for bacterial adhesion sites on intestinal epithelia, alterations of composition and function of the gut microbiome, and an adjuvant-type stimulation of the immune system against pathogenic organisms. In the present study, we investigated the effect of *Bifidobacterium animalis subspecies lactis* (strain Bb12), a human-derived probiotic used extensively in dairy products, on immune activation and intestinal function of young pigs exposed to migrating *A. suum* larvae. Our results showed that Bb12 modulated the liver and intestinal immune response and intestinal physiology without compromising the normal expulsion of the worms. The parasite-induced reduction in intestinal glucose absorption was ameliorated, the tissue eosinophilic response was reduced, and serum and intestinal antibody responses to the worm were enhanced by feeding Bb12.

**Results**

**Quantitative detection of Bifidobacterium animalis subspecies lactis (Bb12) in the intestine**

Bb12-treated pigs born to Bb12-treated sows (T/T) had significantly higher copies per gram (cpg) of the *Bifidobacterium animalis subspecies lactis tuf* gene in proximal colon contents of *Ascaris suum* (*A. suum*)-infected T/T (1.8 ± 0.9 × 10⁵ cpg) compared to non-infected control placebo-treated pigs born to placebo-treated sows (C/C) (0.07 ± 0.07 × 10⁴ cpg; *p* < 0.05) or *A. suum*-infected C/C (0.006 ± 0.004 × 10⁴ cpg; *p* < 0.05) with no difference when compared to non-infected T/T pigs (1.2 ± 0.9 × 10³ cpg) (Figure 1A).

In the second experiment, T/T pigs had higher cpg in fecal samples collected before *A. suum* infection at six weeks of age (0.5 ± 0.1 × 10⁶ versus 0.2 ± 0.01 × 10⁶ cpg; *p* < 0.05) and at 17 days post-inoculation with *A. suum* eggs at nine weeks of age (2.1 ± 0.5 × 10⁶ versus 0.2 ± 0.01 × 10⁶ cpg; *p* < 0.05) with a non significant change in cpg when weaned at 3 week of age (2.56 ± 1.5 × 10⁶ cpg vs 0.2 ± 0.01 × 10⁶ cpg) (Figure 1B).

In the second study, there was also an increase in *Bifidobacterium animalis subspecies lactis tuf* gene cpg in the proximal colon contents of *A. suum* infected Bb12-treated pigs (T/T) (7.3 × 10⁵ cpg) compared to *A. suum*-infected placebo-treated pigs (C/C) (1.4 ± 0.1 × 10⁵ cpg) (*p* < 0.05) at the time of necropsy (Figure 1C).

**Parasite recovery**

*Ascaris suum* L4 are resident in the proximal small intestine at 10 days post-inoculation (p.i.), and are normally expelled distally from day 17 through day 21 of life.
p.i., as part of the normal protective response to infection. This pattern was consistent among all A. suum-infected pigs regardless of treatment with Bb12 or placebo. In the first experiment, three of eight pigs from the T/T and three of six pigs from the C/C treatment groups had <30 L4 in the entire small intestine, and the remaining pigs had the predominant larval burden shifted to the distal two sections of the intestine; the total number of L4 were not significantly different between the two groups (data not shown). In the second experiment, there was no significant difference in the number of L4 detected in the entire small intestine of T/T (2875 ± 746) versus C/C (2249 ± 793) treated pigs at 17 days p.i. (Figure 2).

**Intestinal function**

There was no significant change in resistance in muscle-free mucosa isolated from the jejunum of pigs from any treatment group (Figure 3) in both experiments, which suggested that mucosal resistance was not impaired by parasite infection or feeding Bb12. Sections of muscle-free mucosa from the jejunum of pigs infected with A. suum, however, showed an increased basal I_sc representing the net Cl⁻ ion flux in response to secretagogues like histamine (p<0.05) (Figure 4A), but not to PGE₂ (Figure 4B). The prototypical A. suum-induced prosecretory response to histamine was attenuated significantly (P<0.05) by treatment with Bb12 (Figure 4A), while the responses to PGE₂ remained unchanged (Figure 4B). Infection with A. suum elevated mucosal responses to 5-HT that persisted in pigs fed either placebo or Bb12 (Figure 4C). Jejunal mucosa from pigs infected with A. suum had a characteristic decrease in the absorption of glucose that was attenuated in pigs also fed Bb12 but not placebo (Figure 5).

**Interaction between Bb12 and A. suum at the mRNA level in the jejunum**

A selected gene array was evaluated to test the hypothesis that the Bb12-modulated genes that regulate a local type-2 response and glucose absorption in the jejunum. Forty-three genes were selected to represent categories of relevant cytokines, cytokine receptors, transcription factors, chemokines, mast cell and goblet cell markers, intestinal tight junction, and physiological markers. The jejunum of pigs treated with Bb12 showed no significant change in gene expression compared to placebo-treated pigs except for a <2-fold decrease in expression of IL4 (Figure 6). Infection with A. suum significantly increased (P<0.05) expression of the high affinity Fc receptor of IgE (FCER1A), Resistin-like molecule β (RETNLB), and increased expression of IL-9, IL-13, and mast cell tryptase (TPSAB1) (P<0.1). There was a significantly decreased expression of eosinophil chemotactic...
protein CCL11, and the inducible gene for cyclooxygenase-2, Cox-2 (PTGS2) (P<0.1) compared to control non-infected pigs (Figure 6). The interaction between Bb12 treatment and infection with A. suum showed a significant (P<0.05) up-regulation in RETNLB, IL-13, FCER1A, IGHE, and IL25(IL-17E). There was a significant (P<0.05) decrease in expression of the signature Th1-associated gene IFNG, as well as IL13RA1, IL10, MUC5AC, TLR9, PAR-1 and Treg-derived SOCS3 (Figure 6). Mast cell-associated markers CMA1 and IL3, and the gene for the tight junction protein, TJP1, and PTGS2, also were significantly (P<0.05) down-regulated in A. suum-infected pigs fed Bb12 (Figure 6).

Interaction between probiotic and A. suum at the mRNA level in the colon, mesenteric lymph nodes (MLN) and liver

Gene expression was measured in the proximal colon mucosa where high numbers of Bb12 were detected. Significant down-regulation of gene expression in the proximal colon mucosa was only detected for Forkhead box P3 (FOXP3) in pigs infected with A. suum, and in RETNLB and IL3 in pigs treated with Bb12 (data not shown). The interaction between infection and Bb12 significantly decreased (P<0.05) the expression of TBX21, FOXP3, CMA, IL3, SOCS3, IL25, and TJP1 while only IGHE expression was significantly increased (data not shown). Changes in gene
expression in liver between Bb12 and placebo-treated pigs were compared at 17 days p.i. (Table 1). There was a 2.9-fold reduction in MRC1, a pattern recognition receptor, a 5.7-fold reduction in IL25, a 9.6-fold reduction in STAT6, a 1.8-fold reduction in IL4, and a 2.2-fold reduction in IL1B. No major change in gene expression was detected in the MLN of pigs from the various treatment groups (data not shown).

**Antibody titers to A. suum and Bb12 antigens**

Parasite antigen-specific antibody responses in the serum and intestinal ileal fluid of A. suum-infected and Bb12-treated pigs were measured 21 days p.i. to determine changes in acquired immunity. Infection with A. suum significantly (P<0.05) increased serum IgG1, and IgM anti-Ascaris L3/L4 antibodies compared to non-infected controls in both Bb12 and placebo treated pigs (p<0.05), while the levels of IgA anti-Ascaris L3/L4 antibodies were increased in A. suum-infected pigs treated with Bb12 (T/T-infected) compared to A. suum-infected pigs given placebo (C/C-infected) (Figure 7). In addition, there was a significant increase in IgG1 and IgG2 anti-Ascaris L3/L4 antibodies (P<0.05) in the ileum fluid of A. suum-infected pigs treated with Bb12 (T/T-infected) compared to the other groups. (Figure 8). IgM and IgA anti-Ascaris L3/L4 antibodies in the ileum fluid were not significantly affected by feeding Bb12 (Figure 8).

**Histological and morphometric analysis**

Morphometric measurements were taken from sections of the jejunum of pigs 21 days p.i. to evaluate inflammation and cellular infiltration. There was no significant difference in the average villus height and crypt depth from five representative fields among the treatment groups (data not shown). There was a significant increase, however, in the number of eosinophils in A. suum-infected pigs fed the placebo (6.2 ± 1.6) compared to non-infected control pigs fed the placebo (1.6 ± 0.3) that was inhibited in infected pigs fed Bb12 (2.5 ± 0.3; p<0.05) (Table 2). Goblet cell numbers were not significantly changed, and mast cells were not detected.

**4. Discussion**

Clinical studies suggest that some probiotic bacteria can promote health and prevent disease, however the mechanisms underlying these beneficial effects are not well understood. In this study, the in vivo effect of Bifidobacterium animalis subsp. lactis (Bb12) on intestinal function and immunity against a parasitic nematode was evaluated. Ascaris suum is a persistent and common problem in swine raised worldwide, and the related species in humans, A. lumbricoides (large roundworm) infects an estimated 804 million people. Although infections are common in countries with poor sanitation, ascariasis exhibit a cosmopolitan distribution with sporadic cases also described in developed countries where pigs can be a source of infection. Examination of the immunological and physiological responses to intestinal helminths also has been used to study local and systemic allergic disease.

The analysis of muscle-free segments of jejunum mucosa from Bb12-treated pigs showed no change in mucosal resistance or in net Cl⁻ ion flux in response to PGE2, histamine, 5-hydroxytryptamine (5-HT), acetylcholine, and glucose compared to placebo-treated pigs indicating that the intestinal epithelium is functionally quiescent in pigs fed Bb12. Likewise, mucosal resistance, and secretion in response to PGE₂ remained unchanged in A. suum-infected pigs at 21 days p.i., but the secretory response to histamine and 5-HT were significantly increased. The enhanced mucosal responsiveness to these secretagogues is consistent with the mucosal mast cell hyperplasia that follows infection with A. suum in vivo, and the
functional changes and parasite antigen-induced release of histamine from mast cells isolated from the intestine of A. suum-infected pigs. In the current study, however, a significant increase in mast cell numbers was not found by histological examination of the jejunum of A. suum-infected pigs. Jejunal tissues were fixed in buffered-formalin with the specific objective to evaluate potential morphometric changes due to exposure to Bb12 or the interaction with A. suum infection. Metachromatic staining for pig mast cells, however, is facilitated by acidic fixation in Carnoy’s and low pH staining with toluidine blue to preserve intracytoplasmic granule structure, which was not used in this study. In addition, young confinement-reared pigs have few detectable mast cells in the intestines compared to pigs raised on dirt or after chronic exposure to parasitic infection.

Nevertheless, the increase in basal Iw in intestinal mucosa from A. suum-infected pigs in response to histamine in vitro is comparable to mast cell-dependent increases in fluid in the intestinal lumen that facilitate

Table 1. Type2-associated gene expression in liver of A. suum-infected pigs.

|      | Placebo | Bb12 | Fold change relative to Placebo treatment |
|------|---------|------|------------------------------------------|
| MRC1 | 6.21 ± 0.34a | 7.72 ± 0.37b | -2.9 |
| GATA3 | 8.70 ± 0.18 | 8.96 ± 0.18 | 0.8 |
| IL25 | 11.29 ± 0.36a | 13.81 ± 0.48b | -5.7 |
| FCER1A | 6.67 ± 0.24 | 6.69 ± 0.24 | 0.9 |
| IL13 | 13.36 ± 0.54 | 13.02 ± 0.37 | 1.3 |
| STAT6 | 4.97 ± 0.32a | 8.23 ± 0.80b | -9.6 |
| CCL26 | 4.08 ± 0.88 | 2.77 ± 0.53 | 2.5 |
| IL4 | 12.89 ± 0.24a | 13.75 ± 0.22b | -0.5 |
| IL18 | 10.23 ± 0.30a | 11.38 ± 0.23b | -2.2 |

1Data represent the mean ± SE of Δ Ct for associated Th2 signature genes. Means with different superscript letters are different with p < 0.05.
worm expulsion during the period of self-cure in mice, \(^{30}\) and may be a more sensitive indicator of functional mast cell activity in the pig intestine. \(^{29}\) Concomitant treatment of \(A.\) suum-infected pigs with \(Bb12\) attenuated the hyper-secretory response to histamine (Figure 4) and the reduced sodium (Na)-linked glucose transport that contributes to parasite-induced edema in the intestinal lumen (Figure 5). \(^{31}\) These changes suggest that \(Bb12\) can modulate intestinal function without affecting host protection since parasite clearance from the proximal to distal small intestine was not significantly changed.

It is generally accepted that nematodes like \(A.\) suum are immunogenic and several studies have demonstrated high serum levels of \(A.\) suum antigen-specific antibodies after infection. \(^{32-34}\) In this study, there was a significant parasite-induced increase in serum IgM and IgG1 anti-\(Ascaris\) L3/L4 antibodies at 21 days p.i. that was not affected by feeding \(Bb12\), while \(Bb12\) slightly enhanced IgA anti-\(Ascaris\) L3/L4 antibodies. The IgG1 and IgG2 anti-\(Ascaris\) L3/L4 antibody responses detected in the ileal fluid of \(A.\) suum-infected pigs were marginally but significantly increased in pigs fed \(Bb12\). Probiotics have been shown to enhance humoral immunity to viral infection \(^{35,36}\) and the capacity to increase immune responses to vaccine antigens. \(^{37,38}\) Specific antibody responses to \(Bb12\) antigen were observed only in ileal fluid of \(Bb12\)-treated pigs infected with \(A.\) suum (data not shown), suggesting a synergism that may be due to the enhanced polyclonal expansion of B cells commonly observed during parasitic infection or effects on antigen access and processing in the intestinal mucosa.

Changes in gene expression in the intestinal mucosa observed after infection with \(A.\) suum were characteristic of a type-2 cytokine response with significant up-regulation of \(IL9, IL13\), mast cell-associated activation markers such as \(FCER1A\) and \(TPSAB1\), and goblet cell-specific \(RETNLB\) \((P<0.05)\) (Figure 6). These changes reflect the stereotypical response to intestinal helminths observed in pigs infected with \(A.\) suum \(^{31,39}\) and in mice infected with several different gastrointestinal nematode parasites. \(^{40}\) In the current study, all pigs born to \(Bb12\)-treated sows received oral \(Bb12\) from birth and had high numbers of copies of
the Bb12 tuf gene detected by PCR in the intestinal contents after six and nine weeks of continuous feeding; infection with A. suum did not affect Bb12 copy number in the feces or proximal colon contents after infection. Treatment of pigs with Bb12 induced a significant down-regulation of IL3, TBX21, and RETNLB expression in tissue from the proximal colon, and reduced IL4 gene expression in jejunum (Figure 6); no changes were observed in the MLN of Bb12-treated pigs except for reduced IGHE gene expression (data not shown). These data indicated that Bb12 alone exerts a relatively modest localized immune modulating effect on intestinal mucosa of treated pigs. In contrast, Bb12-treated pigs infected with A. suum showed a significant alteration in the expression of several genes associated with a type-2 response to A. suum (Figure 6). Up-regulation of IL13 was maintained with a concomitant down-regulation of the IL13RA1 chain that is the functional component of the IL-4 receptor complex for IL-13 binding. This finding is consistent with that reported in pigs infected with Trichuris suis but suggests that feeding Bb12 can modulate IL-13 signaling capacity. The expression of IL25 and RETNLB, two genes associated with the regulation of type 2 cytokine-dependent immunity against parasites, were significantly up-regulated (p<0.05) in the jejunum of A. suum-infected pigs fed Bb12. RETNLB, characteristically expressed by goblet cells in the intestine, has been associated with protection against helminths with maximal secretion of RELM beta protein correlated with enhanced Th2 cytokine expression and worm expulsion. IL-25 also has been identified as a critical mediator of type 2 immunity since it is required for regulation of inflammation in gastrointestinal tract. There also was a significant decrease in SOCS3 expression (P<0.05), a major negative feedback regulator of signal transducer, that affects production of the immune regulatory cytokines

**Table 2.** Mean values for eosinophils and goblet cell distribution in jejunum lamina propria.

| Treatment group | Goblet cell | Eosinophil |
|-----------------|------------|------------|
| C/C control     | 13.6 ± 1.2 | 1.6 ± 0.3<sup>a</sup> |
| C/C infected    | 17.9 ± 1.5 | 6.2 ± 1.6<sup>a</sup> |
| T/T control     | 14.6 ± 1.8 | 2.6 ± 0.4<sup>a</sup> |
| T/T infected    | 15.7 ± 1.4 | 2.5 ± 0.3<sup>a</sup> |

Treatment groups: C/C (Ascaris) infected, T/T control, and T/T (Ascaris) infected are compared to C/C control. Statistical significance is indicated by<sup>a</sup> (P ≤ 0.05).

![Figure 8](https://example.com/figure8.png)

**Figure 8.** Anti-Ascaris L3/L4 antibodies in ileum fluid of pigs 21 days post inoculation (p.i.) with infective A. suum eggs. Immunolon plates were coated with A. suum L3/L4 antigen. OD<sub>450</sub> readings are means ± SE, different letters denote differences among treatments after ANOVA (p < 0.05) in (A) IgM, (B) IgG1, (C) IgG2, and (D) IgA.
TGFβ1 and IL-10 through modulation of STAT3 and regulation of IL-17 secreting cells. A significant down-regulation in IL10 gene expression in the jejunum of A. suum-infected pigs fed Bb12 indicated a reduced IL-10 dependent response to infection with A. suum. It should be noted, however, that changes in these regulatory genes could represent a Bb12-induced palliative mechanism that reduced local A. suum induced-eosinophilia in the intestine with no significant effect on parasite expulsion (Table 2). Additional studies that include repetitive and longer exposure to infection, and the effects on memory responses and associated pathology, would be required to test this hypothesis.

Reducing IFN-γ-dependent responses to non-pathogenic bacteria while mounting an appropriate Th1-mediated response to pathogenic bacteria is key to maintenance of physiological homeostasis. The down-regulation of IFNγ and IL10 mRNA expression in Bb12-treated pigs infected with A. suum could represent a beneficial effect of probiotics on intestinal mucosa perturbed by nematode infection. Certain probiotic bacteria reduce pro-inflammatory IFN-γ production independent of IL10. The significant down-regulation of TLR9 in the jejunum of A. suum-infected pigs exposed to Bb12 may also limit bacterial-induced inflammation of the intestinal mucosa.

Protease-activated receptor-1 (PAR-1, F2R) expression is activated by proteases in a variety of tissues including the gastrointestinal tract. Helminth-induced activation of PAR-1 is dependent on IL-13-activated STAT6 signaling pathway independent of IL4. Activation of PAR-1 induces Cl− secretion by intestinal epithelial cells through cyclooxygenase-dependent pathways. Although the mechanism is unknown, immune regulation of PAR-1 and COX2 activity or expression in intestinal mucosa represents novel therapeutic targets given that many reactive allergens and helminths express protease moieties that enhance PAR-1 activation. The significant down-regulation observed in gene expression for both -F2R (5-fold) and PTGS2 (2-fold) in pigs infected with A. suum and fed Bb12 indicates a potentially novel effect of probiotic treatment on intestinal inflammation associated with barrier dysfunction such as is observed in inflammatory bowel disease and celiac disease.

Cytokines directly affect epithelial functions including barrier stability and electrolyte transport across the intestinal mucosa. Various stimuli including cytokines, allergens, and bacterial products enhance mucosal permeability by affecting tight junction (TJ) integrity. Infection of pigs with A. suum or treatment with Bb12 had no effect on epithelial barrier function (resistance) or Cl− secretory responses to acetylcholine and prostaglandin E2. Bb12 treatment, however, abrogated the histamine-induced Cl− secretory response to A. suum. In addition, infection with A. suum or treatment with Bb12 did not change expression for the Cl− channel gene CLCA1 and paracellular permeability genes TJP2, TJP3, and OCLN. There was, however, a 2-fold down-regulation of TJP1 in the jejunal mucosa of Bb12-treated pigs infected with A. suum.

Glucose is the single most abundant actively transported nutrient in human diets. It is transported across the apical or luminal surface via Na-linked glucose transporter SGLT1 (SLC5A1), and the basolateral membrane through the facilitated glucose exchanger GLUT2 (SLC2A2). Trans-epithelial ion transport results in the generation of a negative potential difference (PD) due the active absorption of Na+ ions. The current that is necessary to bring the PD continuously to zero voltage is called the short circuit current (Isc) and is the real time measurement of the net electrogenic transport across an epithelium. Pigs infected with A. suum and treated with Bb12 had a reduction in Isc, indicating a decreased Na-linked transport of glucose. Gene expression for the luminal surface transporter SGLT1 (SLC5A1) did not increase in these pigs suggesting that other mechanisms may be responsible for the reversal of the normal intestinal response to parasite infection. In rodent models of parasite infection, epithelial glucose absorption has been shown to be regulated by macrophages without affecting its expression in the intestine.

While changes in gene expression related to both pro- and anti-inflammatory pathways were relatively minor in pigs treated with Bb12, the effects of feeding Bb12 were more pronounced in pigs following infection with A. suum. This pattern is similar to that observed during infection with Salmonella or E. coli in animals treated with probiotics suggesting that probiotic efficacy is best observed in tissues exposed to an inflammatory stimulus.

We have demonstrated that Bb12 regulates an efficient type-2-dependent response to parasite infection in the intestine by reducing local
intestinal eosinophilic infiltration, the histamine-induced Cl⁻ secretory response, and blocking the inhibitory effects of infection on glucose absorption, while enhancing acquired antibody responses to infection. These data suggest that feeding Bb12 can ameliorate dysregulated type-2 diseases such as allergy and chronic inflammation, and can enhance systemic and local antibody production to certain infections and, by extension, to appropriate immunization strategies.

**Material and methods**

**Animals:** All pigs used in these studies were cross-bred Yorkshire X Poland-China pigs (males and females) born and maintained at the Beltsville Agricultural Research Center swine facility under management conditions approved by the Beltsville Area Animal Care and Use Committee. Six sows were bred by artificial insemination after hormonal synchronization. Pregnant sows were treated with oral administration of either Bb12 probiotic or placebo (maltodextrin vehicle without Bb12) starting the last third of gestation, during nursing, and until weaning of the piglets at three weeks after birth. Three sows received a daily oral dose of 2.2 g of freeze-dried Bb12 (10 billion CFU/g) provided by Christian Hansen, Denmark, as a 100% pure lyophilized product dissolved in 10 mL of sterile PBS, and three additional sows received a daily oral dose of placebo only. Both products (placebo and probiotic) were microbiologically tested throughout the experiment as previously described. Piglets born from Bb12-treated sows also were treated with a daily probiotic dose of 1.1 g (10 billion CFU/g) of Bb12 starting shortly after birth until approximately 2.5 months of age (Treated/Treated = T/T group). Piglets born from placebo-treated sows received a daily dose of 1.1 g of placebo preparation starting at birth until the end of the experiment at approximately 2.5 months of age (Control/Control = C/C group). All piglets were weaned at week three after birth and allocated to individual pens with feed and water ad libitum. To prevent cross-contamination among treatment groups (n = 6-8/group), T/T and C/C pigs were housed in pens at opposite sides of the barn.

In a second study, four sows were also treated with oral administration of either Bb12 probiotic (n = 2) or placebo (maltodextrin vehicle without Bb12, n = 2) as in the first study. Similarly, seven pigs derived from each treatment group were allocated to individual pens to continue the dietary treatment for 2.5 months before collection of samples.

**Inoculation with Ascaris suum**

The acquisition and preparation of infective A. suum eggs, oral inoculation, and management of pigs have been described. In the first experiment, eight piglets from the T/T group and six piglets from the C/C group were inoculated with an oral dose of 2.5 × 10⁴ A. suum eggs, and euthanized at day 21 post inoculation (p.i.). The remaining six animals of the T/T and C/C treatment groups were non-infected controls. Infected pigs were segregated on solid floors from non-infected (control) pigs by a center island that restricted water and particle flow between the opposite ends of the barn. “White spot” liver lesions, an indicator of active migration of A. suum larvae through the liver, were detected in inoculated pigs, but not control non-infected pigs at necropsy. This observation indicated that none of the control pigs were inadvertently infected.

In a second experiment, pigs born from four sows were split into the Bb12 T/T group (n = 7) and placebo C/C group (n = 7) before oral inoculation with 2.5 × 10⁴ A. suum eggs, and euthanized 17 days p.i. to collect A. suum L4 from the small intestine prior to natural expulsion.

**Parasite counts**

Parasite expulsion is indicated by the displacement of L4 from the proximal to the distal small intestine between 17 and 21 days p.i., and six equivalent sections of the small intestine of each pig were examined for L4. Protective immunity to A. suum was determined by comparing the number and location of L4 in the intestines of Bb12-treated versus placebo-treated pigs at 21 days p.i., for pigs in the first experimental group, and 17 days p.i. for pigs in the second experimental group.

**Quantitative detection of Bb12 in fecal samples and intestinal contents**

A 5 g fecal sample was collected in a sterile 50mL conical tube at weaning, three weeks after weaning (pre-
infection) and 17 or 21 days p.i. DNA from the fecal samples or proximal colon contents collected at necropsy was isolated using the QIAamp DNA Stool mini kit (Qiagen, Valencia, CA) as previously described. The abundance of Bb12 in treated-pigs was determined using the primer-probe set against the Bifidobacterium animalis subspecies lactis tuf gene.

**Relative mRNA host gene expression by quantitative real time PCR**

Tissue sections from liver, jejunum, proximal colon, and mesenteric lymph nodes (MLN) collected at necropsy were frozen immediately in liquid nitrogen and stored at −70°C until RNA extraction. Tissue RNA was extracted after homogenization in Trizol reagent (Invitrogen, Gaithersburg, MD), and quantitative real time PCR was performed on cDNA synthesized from each sample using 10 μg of total RNA as previously described. Sequences for all probes and primers selected for real time PCR assays were obtained from the Porcine Translational Research Database (http://www.ars.usda.gov/Services/docs.htm?docid=6065).

Fluorescence signals were processed after amplification and were considered positive if the fluorescence intensity was greater than 20-fold or more than the standard deviation of the baseline fluorescence. Gene expression was normalized based upon a constant amount of RNA and cDNA amplified. Relative quantification of target gene expression was evaluated by comparing Ct values from cDNA processed from non-infected pigs and A. suum-infected pigs at 17 or 21 days p.i. after normalization with the housekeeping gene RPL32. Up- or down-regulation in gene expression is denoted by fold changes in Ct values.

**Measurement of epithelial cell function ex vivo**

Four segments of mucosa (0.126 cm²) were stripped of muscle and mounted in Ussing chambers exposed to 10 mL of Kreb’s buffer. Briefly, agar salt bridges and electrodes were used to measure potential difference. The tissues were short-circuited at 1V (DVC World Precision Instruments, Sarasota, FL) every 50 s, and the short circuit current (Isc) continuously monitored. In addition, every 50 s the clamp voltage was adjusted to 1 V for 10 s to allow calculation of tissue resistance using Ohm’s law. Following a 15min equilibration period, basal Isc representing the net Cl⁻ ion flux before stimulation (baseline), and tissue resistance were measured. After a second 15 min period, concentration-dependent changes in Isc were determined in response to the cumulative addition of 5-hydroxytryptamine (5-HT), acetylcholine (ACH), histamine, or PGE₂ to the serosal side of the intestine. Following re-equilibration, concentration dependent changes in Isc were measured in response to the cumulative addition of glucose to the mucosal side. All responses from treated-tissue segments from control and A. suum infected-pigs were averaged to yield a mean response per treatment group in the first experiment. Mucosal segments from jejunum also were collected in the second experiment for measurement of similar physiological responses.

**Detection of parasite antigen-specific IgA, IgG, and IgM antibodies**

Specific antibodies against A. suum were measured in the first experiment only. ELISA plates (Immunolon, Nunc, Thermo Fisher Scientific, Waltham, MA) were coated with 100 μL of 5 μg/mL of A. suum 3rd to 4th stage larvae (L3/L4) extract or 0.1 μg of lyophilized Bb12 in coating buffer (Sodium Carbonate buffer, pH 9.5). After an overnight incubation at 4°C, plates were washed six times (0.05% Tween 20 in 1X-PBS) and blocked with 0.05% Tween 20, 0.5% BSA in PBS for 30 minutes. Plates were washed and used for testing 100 μL of serum diluted at 1:3,000 and 1:6,000 (0.05% Tween 20 in PBS) or ileum fluid (contents obtained from the terminal 60 cm of pig intestine were centrifuged at 4C and 800 X g for 10 minutes to separate clear fluid from particulates). Test samples were run in duplicates and incubated for two hr at room temperature. After six washes, 100 μL of HRP-conjugated porcine anti-IgM, anti-IgA, anti-IgG1 or anti-IgG2 (Serotec, Raleigh, NC) were added at a 1:10,000 dilution and incubated for one hr. After a final wash, 100 μL of TMB-One substrate solution (Promega, Madison, WI) was added. Total anti-L3/L4 or anti-Bb12-specific antibody isotypes were determined after reading plates with an optical density (OD) at 450nm after 20 minutes. Reaction was stopped by addition of 1M HCl solution.

**Histological and morphometric analysis of jejunum**

Cross-sections of jejunum from the first experiment were fixed in 10% neutral buffered formalin and submitted to the University of Minnesota Diagnostic
Laboratory (St. Paul, MN) for histological analysis. Routine paraffin-embedded tissue blocks were sectioned at 4 μm and stained with hematoxylin and eosin (H&E). Villus height and crypt depth were measured by an observer blinded to treatment. The mean length of 15 well-oriented and representative villi and crypts were measured respectively from each pig. In addition, toluidine blue staining was performed for characterization of cellular components in the intestinal sections. Goblet cells and eosinophils were counted and mean values reported after evaluation of five independent fields under 40X magnification. Measurements were taken using an Olympus BX40 microscope (Olympus Optical Co., LTD, Japan) equipped with a Spot, InSight Color Model 3.20 digital camera and imaging software (Diagnostic Instruments Inc., Sterling Heights, MI).

**Processing of data and statistical analysis**

*Bb12* in feces and the intestinal contents collected at necropsy were determined by quantitative detection of *Bb12-tuf*-gene copies. Data were log-transformed and $C_t$ values compared among dietary treatment groups. Contrast statements were run to compare the effect of parasite infection among treatment groups. Data were transformed to log base to represent bacterial copy numbers per gram (cpg) of *Bb12* using the *tuf* gene as the bacterial marker (mean ± standard error). Statistical results were noted for mean values from each treatment group where any non-identical letters$^{(a,b,c)}$ indicated significant differences at $P \leq 0.05$. The effect of infection with *A. suum* on tissue mRNA gene expression ($C_t$ values) and cell numbers were evaluated using one-way ANOVA analysis and comparing values obtained in samples from *A. suum*-infected and non-infected pigs. Fisher’s LSD post-hoc test was applied to access differences between treatment groups at 17 and 21 days p.i. P$\leq 0.05$ was considered statistically significant for all analyses (Statview 5.0 for Macintosh Abacus Concepts, Berkeley, CA). Differences in mucosal resistance were assessed for both experiments using a one-way ANOVA and concentration-dependent changes in $I_{sc}$ or smooth muscle contractility were assessed by multiple ANOVA. All ANOVA tests were followed by t-tests to evaluate differences between means.

**Conflict of interest statement**

The authors have no conflicts of interest to disclose.

**Financial discloser statement**

This work was funded by USDA project plan 8042-31000-107-00D. The authors have no financial relationships relevant to this article to disclose.

**Acknowledgments**

The authors thank Alyssa Kendall from Diet, Genomics, and Immunology Laboratory for assistance in running some samples for real-time PCR analyses, and Dr. Josh Parker from University of Minnesota for histopathological processing and analysis of pig intestinal sections.

**Funding**

USDA-ARS 8042-31000-107-00D.

**Disclaimer**

The views expressed in this article are those of the authors and do not reflect the official policy or position of the United States Department of Agriculture, Department of Defense, or the U.S. government. Title 17 U.S.C. 105 provides that “copyright protection under this title is not available for any work of the United States Government.” Title 17 U.S.C. 101 defines a United States government work as “a work prepared by a military service member or employee of the United States government as part of that person’s official duties.” This work was prepared as part of the official duties of Drs. Solano-Aguilar, Madden, Dawson, Urban Jr., Lakshman and Mrs. Beshah.

“Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.”

“USDA is an equal opportunity provider and employer”

**References**

1. Limdi JK, O’Neill C, McLaughlin J. Do probiotics have a therapeutic role in gastroenterology?. World J Gastroenterol. 2006;12:5447–57.
2. Kunzelmann K, Mall M. Electrolyte transport in the mammalian colon: mechanisms and implications for disease. Physiol Rev. 2002;82:245–89. doi:10.1152/physrev.00026.2001.
3. France MM, Turner JR. The mucosal barrier at a glance. J Cell Sci. 2017;130:307–14. doi:10.1242/jcs.193482.
4. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Watson A, Wells JM. Intestinal permeability—a new target for disease prevention and
therapy. BMC Gastroenterol. 2014;14:189. doi:10.1186/s12876-014-0189-7.

5. Capaldo CT, Powell DN, Kalman D. Layered defense: how mucus and tight junctions seal the intestinal barrier. J Mol Med (Berl). 2017;95:927–34. doi:10.1007/s00109-017-1557-x.

6. Madden KB, Whitman L, Sullivan C, Gause WC, Urban JF Jr, Katona IM, Finkelman FD, Shea-Donohue T. Role of STAT6 and mast cells in IL-4- and IL-13-induced alterations in murine intestinal epithelial cell function. J Immunol. 2002;169:4417–22.

7. Dhami K, et al. Probiotics in Curing Allergic and Inflammatory Conditions – Research progress and Futuristic vision. Recent Pat Inflamm Allergy Drug Discov. 2017;10:105–18. doi:10.2174/1872213X10666161226162229.

8. Shi HN, Walker A. Bacterial colonization and the development of intestinal defences. Can J Gastroenterol. 2004;18:493–500.

9. Rondanelli M, et al. Using probiotics in clinical practice: Where are we now? A review of existing meta-analyses. Gut Microbes. 2017;8:521–43. doi:10.1080/19490976.2017.1345414.

10. Hayes SR, Vargas AJ. Probiotics for the prevention of Pediatric Antibiotic-Associated Diarrhea. Explore (NY). 2016;12:463–6. doi:10.1080/explore.2016.08.015.

11. Tojo R, Suárez A, Clemente MG, de los Reyes-Gavilán CG, Margolles A, Guimonde M, Ruas-Madiedo P. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. World J Gastroenterol. 2014;20:15163–76. doi:10.3748/wjg.v20.i41.15163.

12. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol. 2013;6:39–51. doi:10.1177/1756283X12459294.

13. Roselli M, Finamore A, Britti MS, Mengheri E. Probiotic bacteria Bifidobacterium animalis SB5 and Lactobacillus rhamnosus GG protect intestinal Caco-2 cells from the inflammation-associated response induced by enterotoxigenic Escherichia coli K88. Br J Nutr. 2006;95:1177–84.

14. O’Hara AM, et al. Functional modulation of human intestinal epithelial cell responses by Bifidobacterium infantis and Lactobacillus salivarius. Immunology. 2006;118:202–15. doi:10.1111/j.1365-2567.2006.02358.x.

15. Takahashi N, et al. Immunostimulatory oligodeoxynucleotide from Bifidobacterium longum suppresses TH2 immune responses in a murine model. Clin Exp Immunol. 2006;145:130–8. doi:10.1111/j.1365-2249.2006.03111.x.

16. Takahashi N, Kitazawa H, Iwabuchi N, Xiao JZ, Miyaji K, Iwatsuki K, Saito T. Oral administration of an immunostimulatory DNA sequence from Bifidobacterium longum improves Th1/Th2 balance in a murine model. Biosci Biotechnol Biochem. 2006;70:2013–7.

17. Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. Curr Opin Biotechnol. 2005;16:204–11. doi:10.1016/j.copbio.2005.02.003.

18. Saavedra JM, Abi-Hanna A, Moore N, Yolken RH. Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. Am J Clin Nutr. 2004;79:261–7.

19. Jang S, et al. Flavanol-Rich Cocoa Powder interacts with Lactobacillus rhamnosus LGG to alter the Antibody Response to infection with the Parasitic Nematode Ascaris suum. Nutrients. 2017;9(10):1113. doi:10.3390/nu9101113.

20. Solano-Aguilar G, et al. Detection of Bifidobacterium animalis subsp. lactis (Bb12) in the intestine after feeding of sows and their piglets. Appl Environ Microbiol. 2008;74:6338–47. doi:10.1128/AEM.00309-08.

21. Karimi O, Pena AS. Probiotics: Isolated bacteria strain or mixtures of different strains? Two different approaches in the use of probiotics as therapeutics. Drugs Today (Barc). 2003;39:565–97.

22. Yan F, Polk DB. Commensal bacteria in the gut: learning who our friends are. Curr Opin Gastroenterol. 2004;20:565–71.

23. Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. Soil-transmitted helminth infections. Lancet. 2018;391:252–265. doi:10.1016/S0140-6736(17)31930-X.

24. Betson M, Nejsum P, Bendall RP, Deb RM, Stothard JR. Molecular epidemiology of ascariasis: a global perspective on the transmission dynamics of Ascaris in people and pigs. J Infect Dis. 2014;210:932–41. doi:10.1093/infdis/jiu193.

25. Miller LA, Colby K, Manning SE, Hoenig D, McEvoy E, Montgomery S, Mathison B, de Almeida M, Bishop H, Dasilva A. Ascariasis in humans and pigs on small-scale farms, Maine, USA, 2010–2013. Emerg Infect Dis. 2015;21:332–4. doi:10.3201/eid2102.140048.

26. Acevedo N, Mohr J, Zakzuk J, Samonig M, Briza P, Erler A, Pomès A, Huber CG, Ferreira F, Caraballo L. Proteinic and immunochemical characterization of glutathione transferase as a new allergen of the nematode Ascaris lumbricoides. PLoS One. 2013;8:e78353. doi:10.1371/journal.pone.0078353.

27. Caraballo L, Zakzuk J, Lee BW, Acevedo N, Soh JY, Sánchez-Borges M, Hossny E, García E, Rosario N, Ansegui I. Particularities of allergy in the Tropics. World Allergy Organ J. 2016;9:20. doi:10.1186/s40413-016-0110-7.

28. Urban JF Jr., Alizadeh H, Romanowski RD. Ascaris suum: development of intestinal immunity to infective second-stage larvae in swine. Exp Parasitol. 1988;66:66–77.

29. Ashraf M, Urban JF, Jr., Lee TD, Lee CM. Characterization of isolated porcine intestinal mucosal mast cells following infection with Ascaris suum. Vet Parasitol. 1988;29:143–58.

30. Sheh-Donohue T, et al. The role of IL-4 in Heligmosomoides polygyrus-induced alterations in murine intestinal epithelial cell function. J Immuno. 2001;167:2234–9.

31. Dawson HD, et al. Localization of multigene expression patterns support an evolving Th1/Th2-like paradigm in response to infections with Toxoplasma gondii and Aasaris...
32. Miquel N, Roepstorff A, Bailey M, Eriksen L. Host immune reactions and worm kinetics during the expulsion of *Ascaris suum* in pigs. Parasite Immunol. 2005;27:79–88. doi:10.1111/j.1365-3024.2005.00752.x.

33. Frontera E, Roepstorff A, Serrano FJ, Gámez A, Reina D, Navarrete I. Presence of immunoglobulins and antigens in serum, lung and small intestine in *Ascaris suum* infected and immunised pigs. Vet Parasitol. 2004;119:59–71. doi:10.1016/j.vetpar.2003.09.022.

34. Eriksen L, Bogh HO, Loftager M, Lind P. Comparative studies of experimental oral inoculation of pigs with *Ascaris suum* eggs or third stage larvae. J Vet Med B Infect Dis Vet Public Health. 2004;51:185–90. doi:10.1111/j.1439-0450.2004.00752.x.

35. Yasui H, Kiyoshima J, Hori T, Shida K. Protection against influenza virus infection of mice fed *Bifidobacterium breve* YIT4064. Clin Diag Lab Immunol. 1999;6:186–92.

36. Qiao H, et al. Immune responses in rhesus rotavirus-challenged BALB/c mice treated with bifidobacteria and probiotic supplements. Pediatr Res. 2002;51:750–5. doi:10.1203/00002640-200206000-00015.

37. Rautava S, Arvilommi H, Isolauri E. Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res. 2006;60:221–4. doi:10.1203/01.pdr.0000228317.7923.d3.

38. Schierack P, Filter M, Scharek L, Toelke C, Tedin K, Haverson K, Lübke-Becker A, Wieler LH. *Bacillus cereus var. toyoii* enhanced systemic immune response in piglets. Vet Immunol Immunopathol. 2007;118:1–11. doi:10.1016/j.vetimm.2007.03.006.

39. Zarlenga DS, Dawson H, Kringle H, Solano-Aguilar G, Urban JF, Jr. Molecular cloning of the swine IL-4 receptor alpha and IL-13 receptor 1-chains: effects of experimental *Toxoplasma gondii*, *Ascaris suum* and *Trichuris suis* infections on tissue mRNA levels. Vet Immunol Immunopathol. 2004;101:223–34. doi:10.1016/j.vetimm.2004.05.003.

40. Shea-Donohue T, Urban JF, Jr. Gastrointestinal parasite and host interactions. Curr Opin Gastroenterol. 2004;20:3–9.

41. Kringle H, Iburg T, Dawson H, Aasted B, Roepstorff A. A time course study of immunological responses in *Trichuris suis* infected pigs demonstrates induction of a local type 2 response associated with worm burden. Int J Parasitol. 2006;36:915–24. doi:10.1016/j.ipara.2006.04.008.

42. Artis D. New weapons in the war on worms: identification of putative mechanisms of immune-mediated expulsion of gastrointestinal nematodes. Int J Parasitol. 2006;36:723–33. doi:10.1016/j.ipara.2006.02.011.

43. Owyang AM, et al. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. J Exp Med. 2006;203:843–9. doi:10.1084/jem.20051496.

44. Artis D, et al. RELMbeta/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. Proc Natl Acad Sci U S A. 2004;101:13596–600. doi:10.1073/pnas.0404034101.

45. Hogan SP, et al. Resistin-like molecule beta regulates innate colonic function: barrier integrity and inflammation susceptibility. J Allergy Clin Immunol. 2006;118:257–68. doi:10.1016/j.jaci.2006.04.039.

46. Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, Hewett DR, McGlorm A, Jolin HE, McKenzie AN. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J Exp Med. 2006;203:1105–16. doi:10.1084/jem.20051615.

47. Cliffe LJ, Humphreys NE, Lane TE, Potten CS, Booth C, Grecins RK. Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. Science. 2005;308:1463–5. doi:10.1126/science.1108661.

48. Kinjyo I, Inoue H, Hamano S, Fukuyama S, Yoshimura T, Koga K, Takaki H, Himeno K, Takaesu G, Kobayashi T. Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-beta 1. J Exp Med. 2006;203:1021–31. doi:10.1084/jem.20052333.

49. Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu BM, Tato C, Yoshimura A, Hennighausen L, O’Shea JJ. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci U S A. 2006;103:8137–42. doi:10.1073/pnas.060066103.

50. Silva SR, Jacsyn JF, Macedo MS, Faquim-Mauro EL. Immunosuppressive components of *Ascaris suum* down-regulate expression of costimulatory molecules and function of antigen-presenting cells via an IL-10-mediated mechanism. Eur J Immunol. 2006;36:3227–37. doi:10.1002/eji.200636110.

51. Sheil B, MacSharry L, O’Callaghan L, O’Riordan A, Waters A, Morgan J, Collins JK, O’Mahony L, Shanahan F. Role of interleukin (IL-10) in probiotic-mediated immune modulation: an assessment in wild-type and IL-10 knockout mice. Clin Exp Immunol. 2006;144:273–80. doi:10.1111/j.1365-2249.2006.03051.x.

52. McCarthy J, et al. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut. 2003;52:975–80.

53. Rachmilewitz D, et al. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology. 2004;126:520–8.

54. Vergnolle N. Review article: proteinase-activated receptors – novel signals for gastrointestinal pathophysiology. Aliment Pharmacol Ther. 2000;14:257–66.

55. Zhao A, Morimoto M, Dawson H, Elyfre J, Madden KB, Gause WC, Min B, Finkelman FD, Urban JF Jr, Shea-Donohue T. Immune regulation of protease-activated receptor-1 expression in murine small intestine during *Nippostrongylus brasiliensis* infection. J Immunol. 2005;175:2563–9.

56. Buresi MC, Buret AG, Hollenberg MD, MacNaughton WK. Activation of proteinase-activated receptor 1...
stimulates epithelial chloride secretion through a unique MAP kinase- and cyclo-oxygenase-dependent pathway. FASEB J. 2002;16:1515–25. doi:10.1096/fj.02-0039com.

57. Resta-Lenert S, Barrett KE. Enteroinvasive bacteria alter barrier and transport properties of human intestinal epithelium: role of iNOS and COX-2. Gastroenterology. 2002;122:1070–87.

58. Zamuner SR, Warrier N, Buret AG, MacNaughton WK, Wallace JL. Cyclooxygenase 2 mediates post-inflammatory colonic secretory and barrier dysfunction. Gut. 2003;52:1714–20.

59. McDermott JR, et al. Mast cells disrupt epithelial barrier function during enteric nematode infection. Proc Natl Acad Sci U S A. 2003;100:7761–6. doi:10.1073/pnas.1231488100.

60. Ceponis PJ, Botelho F, Richards CD, McKay DM. Interleukins 4 and 13 increase intestinal epithelial permeability by a phosphatidylinositol 3-kinase pathway. Lack of evidence for STAT 6 involvement. J Biol Chem. 2000;275:29132–7. doi:10.1074/jbc.M003516200.

61. Youakim A, Ahdieh M. Interferon-gamma decreases barrier function in T84 cells by reducing ZO-1 levels and disrupting apical actin. Am J Physiol. 1999;276:G1279–1288.

62. Zhao A, McDermott J, Urban JF Jr, Gause W, Madden KB, Yeung KA, Morris SC, Finkelman FD, Shea-Donohue T. Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. J Immunol. 2003;171:948–54.

63. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nat Clin Pract Gastroenterol Hepatol. 2005;2:416–22. doi:10.1038/nccpgasthep0259.

64. Turner JR, Cohen DE, Mrsny RJ, Madara JL. Noninvasive in vivo analysis of human small intestinal paracellular absorption: regulation by Na+-glucose cotransport. Dig Dis Sci. 2000;45:2122–6.

65. Seidler U, et al. Molecular mechanisms of disturbed electrolyte transport in intestinal inflammation. Ann N Y Acad Sci. 2006;1072:262–75. doi:10.1196/annals.1326.024.

66. Notari L, et al. Role of macrophages in the altered epithelial function during a type 2 immune response induced by enteric nematode infection. PLoS One. 2014;9:e84763. doi:10.1371/journal.pone.0084763.

67. Riedel CU, Foata F, Philippe D, Adolfsson B, Eikmanns BJ, Blum S. Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-kappaB activation. World J Gastroenterol. 2006;12:3729–35.