Alcohol Consumption by C57BL/6 Mice Is Associated with Depletion of Lymphoid Cells from the Gut-Associated Lymphoid Tissues and Altered Resistance to Oral Infections with Salmonella typhimurium

Don Sibley1,a and Thomas R. Jerrells2

Studies were done to test whether ethanol (ETOH) consumption alters resistance to mucosal and systemic infections by Salmonella typhimurium. S. typhimurium–immune and –nonimmune mice were fed 1 of 3 diets (an ETOH-containing liquid diet, an isocaloric liquid diet equal in volume to that of the ETOH-treated group, or laboratory chow) in a pair-feeding design and were infected orally or intravenously with S. typhimurium. The number of bacteria in spleen and liver and the effect of ETOH feeding and infection on the number of lymphoid cells in the gut-associated lymphoid tissues (GALT) were determined. ETOH feeding resulted in profound loss of GALT lymphoid cells and an increased number of Salmonella organisms in the intestines, liver, and spleen of infected nonimmune, but not of immune, mice. These data show that ETOH consumption in this model impairs host defense mechanisms that control mucosal infections and inhibits the mechanisms that control levels of bacteria in the central organs.

Ethanol (ETOH) abuse by humans is associated with hepatic, neurologic, and immunologic effects and often leads to severe and potentially life-threatening complications. Individuals with a long history of alcohol abuse have some common clinical findings that are germane to the hypothesis that ETOH affects the immune system, which results in an increased incidence of infections [1, 2], certain tumors [3], and autoimmune diseases [3]. The effects of ETOH on the immune system are varied and include the loss of lymphoid cells from the central and peripheral organs [4–8]. ETOH exposure is associated with the loss of nucleated cells from the bone marrow of rats [7, 9] and with the loss of lymphocytes from the thymus, spleen, peripheral blood [6, 10], and mesenteric lymph nodes (MLN) [8] of mice. Flow cytometric analysis of lymphocytes has identified significant losses in T cell populations associated with ETOH consumption by mice. The numbers of immature (CD4+CD8−) T cells of the thymus were reduced by ~66% in mice fed an ETOH-containing diet [10]. Mature CD4+ (helper) and CD8− (suppressor/cytotoxic) T cells of the thymus, spleen [10], and MLN [8] also were reduced by ETOH feeding, although not as severely as were CD4+ and CD8− cells in the thymus.


In addition to the loss of lymphoid cells that is associated with ETOH consumption, several functional deficits associated with ETOH consumption have been identified in cells of the immune system. Thymic lymphocytes exposed to ETOH in vitro demonstrate increased cell death by apoptosis [18, 19]. Mitogen-stimulated T cell proliferation is suppressed by ETOH ingestion, whereas the B cell proliferative response to lipopolysaccharide is unaffected [5, 6, 20]. Likewise, the T cell–dependent antibody responses are suppressed in ETOH-fed animals, while the T cell–independent antibody responses are not affected [5, 6, 21]. In addition, in vitro exposure to ETOH inhibits interleukin-4–induced B cell proliferation [22]. These findings support the proposal [23] that ETOH consumption alters lymphocyte populations and functions, with the T cell components of the immune system being most severely affected. Together, these changes in the immune system may be a factor in the noted susceptibility to infectious diseases associated with ETOH consumption [1, 2, 20, 24].

It has been shown recently that humans who consume alcohol have alterations in the intestinal flora and changes in the
intestinal integrity that cause an increased translocation of endotoxin into the blood stream [25, 26]. It is reasonable to suggest that alcohol abuse results not only in changes in the immune system but also in changes in the gastrointestinal system.

Salmonella typhimurium is a gram-negative, facultative intracellular bacterium that is pathogenic for mice and extensively used as a model for mucosal infection and immunization studies. Resistance to oral infections by S. typhimurium is dependent on a T helper cell type 1 (Th1) cell–mediated immune response, which is characterized by production of interferon (IFN)–γ [27–32] and tumor necrosis factor–α [29, 30, 33–35] and by the subsequent activation of macrophages [27]. It is this cell-mediated immune response by the host that serves to limit the infection to the intestinal mucosa or gut-associated lymphoid tissue (GALT) and to prevent dissemination to the liver and spleen [28].

In addition, mucosal resistance to S. typhimurium after immunization of experimental animals is mediated by the production and secretion of IgA, a T cell–dependent antibody [36]. The class switching of B cells to IgA and the production of secretory IgA are mediated by both Th1 and Th2, but the Th2-type cells are the most effective in this function [37]. Secreted IgA binds to bacteria in the intestinal lumen, which blocks the binding of the bacteria to the epithelium, and thus limits the ability of the bacteria to invade the epithelia. Host resistance to oral infections by S. typhimurium is, therefore, determined by different helper T cell populations in naive and immune animals.

The present studies were designed to test whether ETOH consumption alters resistance to mucosal and systemic infections with S. typhimurium. To this end, nonimmune and immune mice fed an ETOH-containing liquid diet, an isocaloric liquid diet equal in volume to the diet of the ETOH-fed mice, or a solid diet of laboratory chow were infected orally or intravenously with S. typhimurium.

Materials and Methods

Administration of ETOH to experimental animals. ETOH was given as described elsewhere [6] to experimental animals via a liquid diet in a pair-feeding protocol that included animals not given ETOH. In brief, 6–8-week-old female C57BL/6 mice were assigned at random to 3 diet groups that received (1) an ad libitum Lieber-DeCarli liquid diet (catalog number 710262; Dyets, Bethlehem, PA) containing 7% (vol/vol) ETOH and providing 36% ETOH-derived calories; (2) an isocaloric Lieber-DeCarli control diet (catalog number 710029; Dyets) for pair-fed mice, in which each animal received a volume equal to the mean volume consumed by the ETOH diet–treated group; or (3) laboratory chow and water ad libitum.

Mice in the ETOH-containing diet group received 20 mL of fresh diet between 4:30 and 6:00 PM daily. The volume of diet consumed daily by each ETOH-fed mouse was determined, and a volume of the control diet equal to the mean of the volume consumed by the ETOH-fed group from the previous day was fed to each of the pair-fed mice. In most experiments, a group of animals fed laboratory chow and water ad libitum were included to control for the effects of a liquid diet. All animals were provided drinking water ad libitum. Animals were maintained on the appropriate diet for 8 days and tested without allowing terminal withdrawal from ETOH to occur.

Host intestinal flora. The ceca of mice were removed aseptically and homogenized in 5 mL of sterile PBS. Serial 10-fold dilutions of the homogenates were plated in duplicate on MacConkey agar (Difco, Detroit) and blood agar and incubated at 37°C for 24 h in aerobic and anaerobic conditions, respectively. The number of colony-forming units (cfu) of bacteria was determined and averaged for each dilution.

Infection of mice with S. typhimurium. S. typhimurium 986 (a clinical isolate provided by D. W. Niesel, University of Texas Medical Branch, Galveston, TX) was grown in trypticase–soy broth at 37°C, aliquoted into 1-mL vials (2 × 10⁸ bacteria/mL), and stored at −80°C. A fresh aliquot was used for each experiment and was diluted to the appropriate concentration in sterile PBS.

After 3 days of the respective diets, mice were infected orally with a dose of S. typhimurium 986 equal to 1 LD₅₀ for naive mice or 10 LD₅₀ for immune mice. In brief, naive mice were challenged orally by gastric intubation with 5 × 10⁷, and immune mice were challenged with 5 × 10⁶ cfu of Salmonella organisms in 250 μL of sterile PBS immediately after administration of 250 μL of 7.5% sodium bicarbonate.

After 3 days of feeding, naive mice received an intravenous injection (500 cfu/mouse) of live S. typhimurium 986. These mice were killed at 1, 24, and 72 h of infection, and serum samples were obtained for analysis of serum liver enzymes. In addition, liver samples were assayed for cfu of S. typhimurium as described below.

Immunization and challenge of immune mice. Two protocols were used to immunize mice—oral administration of a sublethal dose of viable S. typhimurium and a systemic immunization with heat-killed bacteria administered by intraperitoneal injection.

Immunization with viable bacteria given orally was accomplished as described above, but 5 × 10⁷ organisms were given to each animal.

Intraperitoneal immunizations with heat-killed S. typhimurium 986 were accomplished by administering 2 equal intraperitoneal injections of 10⁷ heat-killed organisms per mouse at 1-week intervals. Bacteria were killed by placing bacteria suspended in PBS into a boiling-water bath for 45 min. The absence of viable bacteria was confirmed by plating each aliquot on trypticase soy agar plates and incubating the plates at 37°C for 48 h.

Resistance of immunized mice to a second infection with S. typhimurium 986 was evaluated by oral (5 × 10⁷ cfu/mouse 14 days after immunization) or intravenous (5 × 10⁷ cfu/mouse 16 days after immunization) infection with live bacteria. Mice were infected by oral inoculation after 3 days of feeding on their respective diet or by intravenous inoculation after 5 days of diet feeding. The mice were killed 72 h after intravenous infections (day 8 of diet) and 5 days after oral infections (day 8 of diet). Livers were obtained to prepare homogenates for determination of cfu of Salmonella organisms, and serum samples were obtained for alanine aminotransferase (ALT) assays.

Determination of bacterial numbers in tissues. The ileum, ce-
cum, MLN, Peyer’s patches (PP), spleen, and liver were removed aseptically and homogenized in sterile saline. In all experiments, care was taken to ensure that the same number of MLN and PP were recovered from each group of mice. Serial 10-fold dilutions of each organ homogenate were prepared in sterile saline and then plated in triplicate on Salmonella-Shigella agar (Difco). The number of bacterial cfu was determined after incubation for 24 h at 37°C.

**Blood alcohol and serum liver enzyme levels.** Blood alcohol levels were determined by obtaining serum samples from individual mice between 9:00 and 10:00 AM and by analyzing the samples with a blood alcohol kit (Sigma, St. Louis). In addition, serum samples were obtained and assayed (according to the manufacturer’s [Sigma] instructions) for the liver enzyme ALT.

**Lymphocyte isolation.** The spleen, MLN, and PP were removed from mice in each treatment group and placed in cold calcium- and magnesium-free Hanks’ balanced salt solution (HBSS; Gibco BRL, Life Technologies, Grand Island, NY). Single-cell suspensions were prepared from the various lymphoid organs by first disrupting the capsule of the PP or MLN by using frosted glass slides or a sterile plunger from a 10-mL syringe and then washing the isolated cells with HBSS. The PP lymphocytes were further purified by gradient centrifugation with a discontinuous percoll gradient (Sigma) to remove the epithelial cells. The mononuclear cell fraction was collected from the interface between the 40% and 70% percoll layers and washed twice with HBSS. The number of lymphocytes recovered from each organ was determined by counting viable cells with a hemocytometer. Flow cytometric analyses of cell populations were done by using monoclonal antibodies (PharMingen, San Diego) and included anti-CD3 for T cells and anti-IgM for B cells.

**Statistical analysis.** All data are reported as mean ± SE of the mean of assays done in triplicate and from a minimum of duplicate experiments. Statistical analyses were performed by using one-way analysis of variance (ANOVA) followed by the Bartlett’s test for homogeneity of variance. All data found to have unequal standard deviations by Bartlett’s test were transformed by log10. The transformed data were then analyzed by using the one-way ANOVA followed by Bartlett’s test. The Bonferroni (Dunn) method for individual comparisons was performed as a post hoc test for all data that conformed to Bartlett’s test. Transformed data that did not conform to Bartlett’s test for homogeneity of variances were compared by Welch’s t test for unpaired data. Significance was defined as P ≤ .05.

**Results**

The data presented here are representative of multiple experiments, and all results were consistent with the data shown.

Serum samples were obtained from both the portal vein and the axillary artery of mice fed an ETOH-containing diet, and serum ETOH levels were assayed to determine the concentrations of ETOH in blood that would reach the central organs, such as the spleen or the intestinal mucosal tissues. Serum ETOH levels in the portal vein (487 ± 147 mg/dL) were significantly (P ≤ .05, Student’s t test) elevated over the blood alcohol levels obtained with blood from the axillary artery (207 ± 56 mg/dL) after 7 days of feeding.

Because of the possible effect of the liquid diets, especially the ETOH-containing diet, on the intestinal bacterial flora, the effects of these diets on the host intestinal flora were tested. The total number of anaerobic cecal bacteria, as determined by cfu assays on blood agar incubated in anaerobic conditions,
and spleen were prepared from animals fed the ETOH-containing or isocaloric control diets (both provided ad libitum), and although the number of enteric bacteria were increased by 3 orders of magnitude in the intestines, bacteria were never detected in liver or spleen homogenates at any time during 7 days of liquid diet feeding.

Lymphocytes from the MLN and PP were isolated 3, 5, and 7 days after feeding mice the various diets. Because of previous studies [8, 10] that showed little to no change in the lymphoid cell numbers in systemic lymphoid organs or MLN after 1 day of ETOH feeding, we did not evaluate early changes in cell populations. The numbers of both MLN and PP lymphocytes recovered from mice fed the ETOH diet decreased through 5 days of feeding (figure 1). The number of MLN and PP lymphocytes recovered from the chow-fed mice and mice pair-fed the control diet was not significantly different ($P > .05$) at days 3, 5, or 7 after oral infection with $S. typhimurium$. Cell numbers obtained for chow-fed and pair-fed groups after 7 days of feeding and infection are presented in figure 1. Except for the IgM+ cell population, which appeared to begin to recover in some mice after 5 and 7 days of feeding, the loss of lymphocytes from the PP of mice fed the ETOH-containing diet followed a time course similar to that observed for the effects of ETOH feeding on the number of lymphocytes in the MLN.

Preliminary data indicate that ETOH consumption increased susceptibility of nonimmune mice to oral infection with $S. typhimurium$, as is evidenced by translocation of bacteria to the spleen and liver [38]. As can be seen in figure 2A, this is a reproducible finding. ETOH-fed, nonimmune mice had a significant ($P < .05$) increase in the number of bacteria in the liver and, of interest, the intestinal tract after 5 days of infection, compared with the number of $Salmonella$ organisms in the livers and intestinal tracts of control mice. The numbers of bacteria in the livers of chow-fed mice 5 days after infection (not shown) were not significantly different ($P > .05$) than the numbers noted in the organs recovered from the pair-fed mice. The increased number of $Salmonella$ organisms in the intestine and liver was first evident in the ETOH-fed mice 3 days after infection and was evident through 7 days of infection (not shown).

The effects of ETOH feeding on the susceptibility of immunized mice to a second challenge infection were tested in ETOH- and pair-fed mice by a second oral infection with 10 LD$_{50}$ of viable $S. typhimurium$. This dose was used on the basis of preliminary data that showed chow-fed mice immunized with a sublethal dose of viable $S. typhimurium$ resisted this challenge dose with little pathologic effects (data not shown). Further, these preliminary data also showed that the chow-fed animals had undetectable levels of $S. typhimurium$ in the intestinal tract, liver, and spleen after 7 days of infection. Mice in both groups immunized with a nonlethal, oral dose of $S. typhimurium$ 986 showed a significant ($P < .05$) increase in the number of $S. typhimurium$ in the cecum 5 days after an oral infection with 10 LD$_{50}$. The number of $Salmonella$ organisms in the intestinal...
Figure 3. Effects of ethanol (ETOH) feeding on the numbers of Salmonella organisms recovered from the liver and on serum levels of the liver enzyme alanine aminotransferase (ALT) after intravenous infections with Salmonella typhimurium. For mice fed an ETOH-containing liquid diet or pair-fed a control diet, colony-forming units (cfu) of Salmonella organisms (A) and serum levels of ALT (B) were determined 1, 24, and 72 h after intravenous infection with viable S. typhimurium (500 cfu/mouse), as described in Materials and Methods. Data are mean ± SE of triplicate determinations from 3 separate experiments. Statistical analyses were done by using Welch’s t test for unpaired data. * P < .05, compared with the appropriate values for pair-fed mice.

Extracts of the ETOH-fed mice, however, was not greater than the number in the ETOH diet–fed, nonimmune mice 5 days after receiving an oral inoculum of 1 LD₅₀ (figure 2A). It was rare to isolate Salmonella organisms from the ileum after 5 days of infection (not shown); however, the bacteria persisted in the cecum after 5 days. Although the number of S. typhimurium in the intestines of ETOH-fed mice was significantly higher than that in the pair-fed mice, there was no significant difference in the number of bacteria isolated from the livers of mice in each group (figure 2B). In the experiments described here, there was no difference between the data obtained from the pair-fed mice and those obtained from mice fed laboratory chow ad libitum (not shown).

To test the effects of an ETOH-containing diet on systemic resistance to Salmonella infections, we gave mice from all feeding groups an intravenous injection of 500 cfu of S. typhimurium per mouse—a sublethal dose—after 5 days of feeding. At various times (1–48 h) after infection, the number of Salmonella bacteria in the livers was determined. In addition, serum samples were obtained and analyzed for levels of liver enzymes as an indirect measurement of hepatic injury (figure 3). The number of Salmonella organisms isolated from the livers 1 h after intravenous infection were not different for ETOH-fed mice and mice pair-fed a control diet (figure 3A). At 24 and 72 h after the intravenous infection, the numbers of Salmonella organisms recovered from the livers of mice fed the ETOH-containing diet were significantly (P < .05) greater than those recovered from the pair-fed (figure 3A) and chow-fed (not shown) mice. The levels of serum ALT from both ETOH-fed and pair-fed mice were at control levels 1 h and only slightly elevated above those values 24 h after intravenous infections (figure 3B). By 72 h of infection, the ALT levels of animals in the ETOH-fed and pair-fed groups were significantly (P < .05) higher than those of noninfected control mice (54 ± 28 U/L). In addition, after 72 h of infection, the serum ALT level was significantly higher in ETOH-fed mice than in pair-fed mice.

To determine the effects of ETOH feeding on systemic immunization with heat-killed bacteria (which is mediated by antibody) as opposed to mucosal immunity after oral infection by viable bacteria (which is mediated by cellular mechanisms), mice in the various feeding groups were immunized with 10⁷ heat-killed S. typhimurium 1 week apart. Two weeks after the last injection, the mice were fed either the ETOH-containing or control diet. These ETOH-fed and pair-fed mice, as well as a group of nonimmunized chow-fed mice, were infected orally with 10 LD₅₀ or by intravenous injection of 500 cfu of S. typhimurium as described above.

Seventy-two hours after intravenous infection and 5 days after oral infection, the number of bacteria in the liver and serum levels of ALT were determined. The number of bacteria in the livers of the immunized ETOH-fed mice after the intravenous infections (figure 4A) was similar to the bacterial counts noted in the nonimmune ETOH-fed mice infected intravenously (figure 4A), which indicated a lack of systemic immunity in the mice immunized with heat-killed bacteria. The serum levels of ALT 72 h after intravenous infections of the ETOH-fed mice immunized with heat-killed Salmonella organisms also were correspondingly higher than the levels in the pair-fed and chow-fed control mice. As expected, all groups of mice had higher ALT levels than uninfected chow-fed mice (54 ± 28 U/L) and uninfected ETOH-fed mice (123 ± 15 U/L).
Figure 4. Effects of ethanol (ETOH) feeding and systemic immunization with heat-killed *Salmonella typhimurium* on resistance to subsequent oral and intravenous infections with *S. typhimurium*. Mice were immunized by intraperitoneal injections with heat-killed *S. typhimurium* 2 weeks before receiving an ETOH-containing diet (ETOH fed) or a control diet (pair fed), as described in Materials and Methods. A group of nonimmunized mice were maintained on laboratory chow and water ad libitum (chow fed). Colony-forming units (cfu) of *Salmonella* organisms in the livers (A) and the serum level of alanine aminotransferase (ALT) (B) were determined for mice from each diet group infected with an oral dose of 10 LD<sub>50</sub> or an intravenous dose of 500 cfu per mouse of *S. typhimurium*. Data are mean ± SE of data from 5 mice per group. Statistical analyses were done by using Welch’s *t* test for unpaired data. *P* < .05, compared with the data from the chow-fed animals.

Although the numbers of *Salmonella* organisms in the livers of immunized mice were confirmed by relatively low serum ALT levels (figure 4B).

Discussion

Several studies, both experimental and epidemiologic, support the suggestion that ETOH consumption by experimental animals and humans results in increased susceptibility to infectious diseases [1, 2, 20, 24], including intracellular bacteria [38–40]. It is important to note that this includes infection of the gastrointestinal tract [40]. For the most part, the experimental model systems have been done with the use of microorganisms that produce a systemic infection [38–42]. The data presented in this paper show that ETOH consumption by mice results in alterations in mucosal host defense mechanisms that ultimately result in an increased susceptibility to oral infections by *S. typhimurium*. Of interest, the consumption of ETOH did not affect the ability of mice immunized by a sublethal oral infection to resist a second oral infection. Further, it is apparent from the data presented here that ETOH-fed mice were not protected from a systemic infection—which was administered by an intravenous injection—by immunization with heat-killed organisms.

Other researchers have shown that control of *S. typhimurium* to prevent systemic infections is a function of a Th1-type immune response, especially activation of macrophages by IFN-γ in GALT [29, 31–33]. Together, our data support the suggestion that ETOH affects the cell-mediated immune response, most notably the Th1-type response, to *S. typhimurium*.

Although there is no direct evidence to support the suggestion that ETOH consumption by experimental animals results in a suppression of the Th1-type cellular immune response, a number of observations indirectly support this idea. First, Waltenbaugh et al. [43] interpreted data obtained from ETOH-fed mice immunized with synthetic antigens to suggest that Th1-type responses, as measured by delayed-type hypersensitivity, are suppressed in ETOH-fed mice, but Th2-type responses, as determined by antibody production, are not affected or perhaps enhanced.

There have been few studies designed to investigate the effects of ETOH on the gastrointestinal tract [11, 25, 26] and even fewer that have addressed immune responses to infectious organisms [38, 40]. Overall, these studies have established that ETOH does affect the gastrointestinal tract, and there are some data published that have led us to suggest that ETOH consumption can alter the ability of a host to limit gastrointestinal infections [40]. This study was done to establish whether ETOH consumption by experimental animals in a well-described feeding protocol alters infection with the bacterium *S. typhimurium*, a true mucosal pathogen of mice, and to begin to elucidate the mechanisms that may be involved.

It is clear from the data that ETOH consumption dramati-
cally increases susceptibility of nonimmune mice to oral infection with S. typhimurium. This was evidenced by the large numbers of bacteria in the livers and spleens of infected mice. We interpret these data to indicate that there is increased translocation of bacteria from the gastrointestinal tract to the blood stream and, ultimately, to the liver and spleen. Again, we suggest that this is because of changes in the Th1-type immune responses and macrophage activation at the mucosal level. Because of the requirement for CD4+ and CD8+ T cells to prevent translocation of S. typhimurium to the blood stream [44], the loss of lymphoid cells from the systemic lymphoid organs [5, 6, 10] and GALT ([8] and this report) and the impairments in T cell function [5, 6, 20] associated with ETOH consumption would also affect the ability of ETOH-fed mice to control the infection.

On the other hand, ETOH did not affect the ability of mice immunized with an oral sublethal dose of S. typhimurium to resist an oral rechallenge. Immunity to oral infection with this bacterium is mediated by IgA that is released into the gut and blocks the ability of the bacteria to infect the gut tissue [36]. Although both Th1- and Th2-type lymphocytes can help class switch B cells to IgA production, the Th2-type T cell is the most effective and important for this process [37]. It is reasonable to suggest on the basis of the data presented here that the IgA memory B cells in GALT were not affected in the ETOH-fed mice and that a recall immune response contributed to the resistance of these mice to oral rechallenge with homologous bacteria. Experiments are in progress to test this hypothesis.

Another explanation for the increased numbers of bacteria in the liver and spleen of ETOH-fed mice is that translocation occurs at the same rate in all groups of mice, and only in the ETOH-fed animals are the immune mechanisms operative in these organs to control the growth of the bacteria affected. This possibility is supported by the observed increased susceptibility of ETOH-fed mice to intravenous challenge with S. typhimurium, which was similar to the effects of ETOH feeding on systemic infections with Listeria monocytogenes [42]. We do not favor this possibility as the sole reason for the observed effects of ETOH on the basis of work that shows the importance of immune responses at the gut level for control of translocation of S. typhimurium from the gastrointestinal tract [32]. We would indeed propose that the suppression of systemic immune responses further impairs the ability of the ETOH-fed mice to control the infection and enhances the pathologic effects of the mucosal infection by S. typhimurium.

Another finding of note presented in this report is the increase in numbers of S. typhimurium in the gut of both naive and immune animals associated with ETOH consumption. S. typhimurium invades the mucosal epithelium of the ilium and divides at this site [45]. A possible explanation for the increased numbers of Salmonella bacteria in the gastrointestinal tract of the ETOH-fed mice is that nonspecific host factors, such as components of the mucus, production of cytokines by the epithelial cells, and many other factors important in host defenses against S. typhimurium [33, 35, 46], are altered by ETOH feeding. We have no direct evidence to address this intriguing question but the increased bacterial burden of the ETOH-fed animals is of potential importance for understanding the increased susceptibility to infection noted in this model system and is currently under study in our laboratory.

Also of importance is the observation that some component of the liquid diet resulted in an increase in the enteric bacterial flora of the treated mice. The fact that this large increase in enteric bacteria did not result in translocation of any bacteria to systemic organs, at least at the level of detection of the assays used in this study, shows that the effects of ETOH described in this study are restricted to bacteria that have the ability to invade the mucosal epithelium and translocate through the epithelium and PP. This rules out a nonspecific damage to the mucosal integrity by ETOH, as described by others, for stomach integrity [11].

It is interesting to note that immunization with heat-killed bacteria, which induces a Th2 immune response [47, 48], inhibited translocation of bacteria from the gut but had no effect on bacteria administered intravenously. Some reports show that antibodies can protect against infections with Salmonella species [49]; however, it is not clear why the antibodies produced in response to the immunization with heat-killed bacteria did not protect in the present study. This may be related to the effects of ETOH on macrophage functions, as described by others [15–17, 39], and to our unpublished observations that show that macrophages from ETOH-fed mice are unable to respond to IFN-γ to control the intracellular growth of S. typhimurium.

Overall, the results of this study provide a model to explore the mechanisms that result in an increased susceptibility to pathogenic bacteria that invade the gastrointestinal tract.

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References

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