Gut-associated lymphoid T cell suppression enhances bacterial translocation in alcohol and burn injury

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Gut-associated lymphoid T cell suppression enhances bacterial translocation in alcohol and burn injury. Am J Physiol Gastrointest Liver Physiol 282: G937–G947, 2002. First published January 30, 2002; 10.1152/ajpgi.00235.2001.—The mechanism of alcohol-mediated increased infection in burn patients remains unknown. With the use of a rat model of acute alcohol and burn injury, the present study ascertained whether acute alcohol exposure before thermal injury enhances gut bacterial translocation. On day 2 postinjury, we found a severalfold increase in gut bacterial translocation in rats receiving both alcohol and burn injuries compared to the animals receiving either injury alone. Whereas there were no demonstrable changes in intestinal morphology in any group of animals, a significant increase in intestinal permeability was observed in ethanol- and burn-injured rats compared with the rats receiving either injury alone. We further examined the role of intestinal immune defense by determining the gut-associated lymphoid (Peyer’s patches and mesenteric lymph nodes) T cell effector responses 2 days after alcohol and burn injury. Although there was a decrease in the proliferation and interferon-γ by gut lymphoid T cells after burn injury alone; the suppression was maximum in the group of rats receiving both alcohol and burn injuries. Furthermore, the depletion of CD3+-cells in healthy rats resulted in bacterial accumulation in mesenteric lymph nodes; such CD3+-cell depletion in alcohol- and burn-injured rats furthered the spread of bacteria to spleen and circulation. In conclusion, our data suggest that the increased intestinal permeability and a suppression of intestinal immune defense in rats receiving alcohol and burn injury may cause an increase in bacterial translocation and their spread to extraintestinal sites.

T lymphocyte; infection immunity bacteria; inflammation; shock

DESPITE ADVANCES IN THE INTENSIVE care units, infection remains the leading cause of multiple organ failure following burn, trauma, and hemorrhage (14, 17). The management of these patients becomes much more difficult when these injuries are accompanied with prior alcohol consumption (27, 33, 37, 46, 49). Nearly 50% of all burn-trauma patients are shown to have alcohol in their blood. A number of previous studies have shown that prior alcohol exposure contributes significantly to the complications associated with burn and trauma (27, 33, 46). These patients exhibit higher incidence of infection and are likely to die compared with the patients with no alcohol exposure (39, 53). Similarly, alcohol consumption by experimental animals also results in increased susceptibility to infection following burn injury (7, 16, 28, 39). The mechanism of such alcohol-mediated increased incidence of infection and associated mortality remain unknown.

A few studies have indicated that hospitalized patients often succumb to opportunistic pathogens originating from their own gastrointestinal (GI) tracts (11, 12, 40, 50). The GI tract is normally very effective in keeping bacteria in the lumen. However, injury conditions such as burn or trauma may disrupt the normal intestinal barrier functions and thus may allow increased bacterial infiltration to mesenteric lymph nodes (MLN) and other extraintestinal sites including spleen, liver, lung, and blood (11–13, 40, 48). This passage of bacteria from the GI tract to extraintestinal sites has been termed bacterial translocation. Three major factors could contribute to bacterial translocation: 1) physical disruption of mucosal barrier, 2) intestinal overgrowth of bacteria, and 3) suppression of the immune defense.

A major complication in burn patients is their inability to mount an appropriate host response to invading pathogens (3, 5, 6, 14, 23, 29, 36, 41, 51). Both clinical and experimental evidence suggests that burn injury results in a state of immunosuppression. This suppression is characterized by a decrease in splenic and peripheral blood T cell proliferation (5, 6, 23, 29, 41, 51) and macrophage dysfunction (36). Similar changes are noted in cells of the immune system after acute and chronic alcohol consumption (10, 24–26, 54, 56). Although clinical evidence showing enhanced morbidity and mortality in burn patients with prior alcohol exposure compared with patients with no alcohol exposure is overwhelming, only a few studies have been

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carried out to address the underlying mechanism. These studies have shown that acute alcohol exposure before thermal injury produced a greater suppression of mitogen-induced splenic-lymphocyte proliferation, serum immunoglobulin levels, and neutrophil chemotaxis (28). In addition, studies by Napolitano et al. (39) have suggested that chronic alcohol exposure resulted in damage of both gut villi and submucosal region. More recent studies, including our own (7, 16), suggest that mice receiving low doses of alcohol before burn injury exhibit impaired delayed-type hypersensitivity, splenic T cell proliferation, interleukin (IL)-2 production, and enhanced susceptibility to infection. The damage in gut as shown by Napolitano et al. (39) may contribute to enhanced bacterial translocation. However, the process of infection involves not only the passage of bacteria from the GI tract to extraintestinal sites but also survivability of translocated bacteria in the extraintestinal sites. Thus the host immune defense becomes a much more critical component in the bacterial translocation process. In the present study, we examined gut lymphoid [mesenteric and Peyr’s patches (PP)] T cell proliferation and interferon (IFN)-γ production to determine whether alterations in intestinal T cell effector functions are responsible for enhanced bacterial translocation.

MATERIALS AND METHODS

Animals and reagents. Adult male Sprague-Dawley rats weighing 225–250 g were obtained from Harlan (Indianapolis, IN). The rats were maintained in accordance with the guidelines set forth by Loyola University Chicago Medical Center Animal Care and Use Committee. Concanaevalin A (ConA) was purchased from Sigma (St. Louis, MO). Nylon wool was obtained from Polysciences (Warrington, PA). Reagents for cell culture were obtained from Biowhittaker (Walkersville, MD). Reagents for bacterial culture were purchased from Difco (Detroit MI). [3H]lactulose and [14C]mannitol were purchased from American Radiolabeled Chemicals (St. Louis, MO).

Rat model of acute alcohol and burn injury. We used the rat model of acute alcohol and burn injury as suggested by Kawakami et al. (28). Rats were randomly divided into four experimental groups: 1) sham + saline, 2) sham + alcohol, 3) burn + saline, and 4) burn + alcohol. In alcohol-treated groups, the levels of blood alcohol equivalent to 90–100 mg/dl were achieved by gavage feeding of 5 ml of 20% alcohol in saline. In some experiments, animals were also gavaged with 40% ethanol to achieve a blood alcohol level of 240–250 mg/dl to determine whether the absolute blood alcohol level was a determinant of the outcome. In saline groups, animals were gavaged with 5 ml saline. Four hours after alcohol gavage, all animals except the control group were anesthetized with pentobarbital sodium (65 mg/kg). Hairs were shaved from their dorsal body surface. For burn procedure, animals were transferred into a template, which was fabricated to expose 25–30% of the total body surface area. Animals were then immersed in a hot water bath (95–97°C) for 10 s. Sham-burn animals were subjected to identical anesthesia and other treatments, except they were immersed in lukewarm water. The animals were dried immediately and given fluid resuscitation with ~10 ml physiological saline. Animals were allowed to recover from anesthesia and were returned to their cages. Animals were allowed food and water ad libitum.

Measurements of bacterial translocation. Two days (~48 h) after alcohol and burn injury, animals were anesthetized and killed. The abdominal cavity was exposed under aseptic conditions. Mesenteric lymph nodes, spleen, and blood were collected. The organs were weighed and homogenized in sterile glass tissue grinders (Fisher Scientific). Equal volume of blood (10 µl) or tissue homogenate (10 µl) from various experimental groups was cultured separately on Tryptic soy agar (Difco) plates. The agar plates were incubated for 24–48 h at 37°C for the growth of bacteria. Bacterial colony-forming units (CFU) were counted. If the plates did not show any bacterial growth up to 48 h, the organ was considered negative for the presence of bacteria.

Histochemical analysis of intestine. As described previously (13, 48), 1-cm-long pieces of small intestine were fixed in 10% formalin in PBS for 4 h and embedded in paraffin. These were then cut (4–5 µm) and mounted on glass slides. Using the standard hematoxylin-eosin staining procedure, the intestine sections were observed under the light microscope for changes in intestinal morphology and later photographed. For electron microscopy, intestinal rings (0.5-cm long) were fixed in a 4% glutaraldehyde solution and transferred to the Loyola University Medical Center Core Imaging Facility (www.meddean.luc.edu/cif) for further processing. Briefly, tissues were washed in buffer; postfixed in 1% osmium tetroxide and dehydrated in a graded series of alcohol followed by propylene oxide. Spurr low-viscosity resin (Electron Microscope Sciences, Ft. Washington, PA.) was used for infiltration and embedding. Blocks were polymerized at 56°C overnight. Sections were cut at 1 µm and stained with 1% toluidine blue for light microscopic evaluation and orientation followed by thin sectioning at 80 nm with a diamond knife. Sections were stained with 5% uranyl acetate and Reynolds’s lead citrate, analyzed at 75 kV on a Hitachi H-600 transmission electron microscope, and photographed (×20,000 magnification).

Intestinal permeability. Rats’ right femoral arteries were cannulated under anesthesia, using PE-50 tubing filled with heparin saline (10 U/ml), and midline laparotomy was performed (48). Renal artery and renal vein in both kidneys were ligated. A 20-cm-long ileum was isolated without damaging intestinal and mesenteric structures, and PE-10 tubing was cannulated into the isolated ileum from the proximal end. As described previously (48), trace quantities of [3H]lactulose and [14C]mannitol were mixed in 1 ml of saline and injected into the isolated ileum through the tubing. The abdominal wall was closed with suture. One milliliter of blood was collected at 80 min from a cannulated femoral artery. Plasma was collected and mixed with 5 ml of liquid scintillant (ICN Biomedical). [3H]lactulose and [14C]mannitol radioactivity were counted by using a two-channel liquid-scintillation counter (Beckman Coulter) and expressed as cpm.

Cell preparation. Rats were anesthetized, and via midline incision, the intestine was exposed. PP and MLN were removed aseptically. Isolated PP and MLN were gently crushed to prepare a single-cell suspension in Hanks’ balanced salt solution (HBSS) supplemented with 10 mM HEPES and 50 µg gentamicin/ml. To obtain pure T cell preparation, cell suspensions were incubated with nylon wool-packed columns. These columns were preincubated with HBSS supplemented with 10 mM HEPES, 5% fetal calf serum (FCS), and 50 µg gentamicin/ml. The columns containing cells were
incubated at 37°C for 50–60 min. T cells were obtained by eluting the columns with 30–40 ml HBSS at a flow rate of 1 drop/s.

T cell proliferation. For the measurements of T cell proliferation, mixed MLN and PP cells and isolated T cells were resuspended in RPMI 1640 supplemented with l-glutamine (2 mM), 2-mercaptoethanol (50 μM), HEPES (10 mM), gentamicin (50 μg/ml), and FCS (10%) at a density of 5 × 10^6 cells/ml. One hundred microliters of the cell suspensions were added to the wells of a 96-well plate (5). The cells were cultured at 37°C and in 5% CO2 in the presence or absence of ConA (5 μg/ml). After 66 h of culture, 0.5 μC [3H]thymidine were added to each well containing the cells. After an additional 6 h incubation, cells were harvested using a PHD cell harvester (Cambridge Technology, Watertown, MA). The incorporation of radiolabeled thymidine was quantified using a liquid-scintillation counter, and the data are expressed as dpm.

T cell IFN-γ production. Nylon wool-purified T cells (5 × 10^6/well) were cultured in a 96-well plate for 48 h in the presence of ConA at 37°C in the presence of 5% CO2. Supernatants were harvested 48 h after culture. IFN-γ in the supernatants was measured using ELISA kits (Biosource International).

Depletion of T cells. Rats were intraperitoneally injected with a mixture of anti-CD4 (OX8; 5 mg/kg) and anti-CD8 (OX38; 5 mg/kg) to deplete the CD4 and CD8 T cells. These doses of antibodies were used to deplete CD4 and CD8 cells in previous studies (1). Immediately after the administration of antibody, rats were gavaged with saline or alcohol and underwent sham or burn injury. Rats were killed on day 2 postinjury. Blood was drawn via cardiac puncture. MLN and spleen were aseptically removed. The depletion of T cells was determined in circulation, spleen, PP, and MLN by counting CD3-positive cells using FITC-labeled antibodies to CD3 and flow cytometry.

Statistical analysis. These data, wherever applicable, are presented as means ± SE and were analyzed using ANOVA statistical program (Statistical Package for Social Sciences Software program, version 2.0; SigmaStat). A P value < 0.05 between groups was considered as statistically significant.

The experiments described here were carried out in adherence to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

RESULTS

Circulating blood ethanol levels. Two separate groups of rats (4 in each group) were gavaged with a single dose of 5 ml of 20 and 40% alcohol. After 4 h, rats were killed by CO2 inhalation. Blood was drawn via cardiac puncture, and serum levels of ethanol were measured. Administration of 5 ml of 20% alcohol resulted in a blood ethanol level in the range of 90–100 mg/dl, whereas the administration of 5 ml of 40% gave a blood ethanol level in the range of 240–250 mg/dl. An additional series of experiments was performed to monitor the clearance of alcohol from the bloodstream (Fig. 1). Rats were killed at 0.5, 2, 4, 6, 8, and 24 h after alcohol administration, and their blood alcohol levels were measured. An increase in blood ethanol levels was noted within 30 min of alcohol administration. As shown in Fig. 1, Rats gavaged with 5 ml of 20% alcohol achieved blood ethanol levels in the range of 170–180 mg/dl, whereas 5 ml of 40% alcohol resulted in values of 380–400 mg/dl within 30 min of alcohol administration. Approximately 70–75% of the circulating alcohol was metabolized within 8 h after administration and 100% after 24 h. The sensitivity of the ethanol detection kit was 10 mg/dl; thus values <10 mg/dl were considered negative.

Determination of bacterial number. Bacterial numbers were determined by culturing blood and homogenates prepared from MLN and spleen of various experimental groups. As shown in Fig. 2, the number of bacteria recovered in the MLN of sham animals was (1.5 ± 0.7 CFU). Slightly more bacterial counts (3.8 ± 1.1) were noted in MLN of sham animals receiving a single dose of alcohol. The number of bacteria recovered from MLN of burn animals (10 ± 2.1) was significantly higher (P < 0.05) compared with MLN of animals receiving either sham or sham and alcohol injury. A severalfold increase in bacteria (72 ± 17) was recorded in animals subjected to combined alcohol and burn injury. No bacterial growth was noted in the blood and in the homogenates prepared from spleens of animals from any experimental group.

Morphological analysis of intestine. Animals were killed 2 days after alcohol and burn injury, and the morphological analyses of intestines were carried out using both light and electron microscopy. Photomicrographs of intestine from light microscopy are presented in Fig. 3, A (sham + vehicle), B (sham + ethanol), C (burn + vehicle), and D (burn + ethanol). There was no
Evidence of any substantial damage to the villi or submucosa of the intestine of animals following alcohol exposure and burn injury (Fig. 3C) compared with sham animals (Fig. 3, A or B). To further confirm these observations, intestines from various experimental groups of animals were examined under an electron microscope, and results from these analyses are presented as Fig. 3, a (sham + vehicle), b (sham + EtOH), c (burn + vehicle), and d (burn + EtOH). Similar to the light microscopy, there were no demonstrable differences in the morphology of the intestines of alcohol- and burn-injured rats compared with the intestines of rats receiving either burn + vehicle or sham injury. As can be seen in the representative electron micrographs, there was no difference in the tight junctions (shown by arrows) in the intestines of alcohol- and burn-injured rats (Fig. 3d) compared with the intestines of burn alone (Fig. 3c) as well as the intestines of sham-injured rats regardless of their treatment (Fig. 3, a and b).

Lactulose and mannitol permeability. Figure 4 shows increases in plasma concentrations of lactulose and mannitol after their infusion into the intestinal lumen. Increases in plasma lactulose and mannitol concentrations after their transfer from the intestinal lumen were not significantly different in the sham group regardless of their treatment. However, a significant ($P < 0.05$) increase in plasma levels of lactulose and mannitol was observed on day 2 in burn rats receiving vehicle alone compared with sham animals. A further increase in plasma levels of both lactulose and mannitol was recorded in burn-injured rats receiving ethanol. However, the increase in plasma lactulose was not found significantly different between burn and burn + ethanol groups. In contrast, the plasma mannitol levels in the burn + ethanol group of rats were significantly ($P < 0.05$) higher compared with those observed in burn-alone groups.

Effect of alcohol exposure on intestinal T cell proliferation after burn injury. We determined ConA-mediated T cell proliferative responses both in mixed as well as in nylon wool-purified MLN and PP cells. As shown in Fig. 5, there was no change in the proliferation of ConA-mediated mixed PP (Fig. 5A) and MLN (Fig. 5B) cells obtained from sham animals receiving alcohol compared with sham rats receiving saline. The proliferation of PP and MLN cells obtained 2 days after burn injury was significantly decreased compared with the cells derived from sham group of animals (Fig. 5, A and B). The proliferation was further suppressed in PP and MLN cells derived from rats receiving both alcohol and burn injuries. In subsequent experiments, we purified T cells from PP and MLN using nylon wool columns as described in MATERIALS AND METHODS. Proliferative responses in nylon wool-purified T cells were determined to delineate whether or not the observed ConA-mediated proliferative disturbances in PP and MLN T cells were due to the presence of other accessory cells. Results from these experiments are shown in Fig. 5, C and D. Similar to mixed PP and MLN cells, we found significantly more suppression in the proliferation of isolated T cells from PP (Fig. 5C) and MLN (Fig. 5D) of rats receiving both alcohol and burn injury compared with the T cells derived from rats receiving either injury alone. These results suggest that ethanol exposure before burn injury exacerbates the suppression of intestinal T cells.

Additional experiments were carried out to demonstrate whether or not the blood-alcohol level at the time of injury impacts the degree of suppression of intestinal T cell proliferation. Rats were gavaged with 5 ml of 40% alcohol to achieve a blood alcohol level in the range of 240–250 mg/dl before sham or burn injury. As shown in the Fig. 6, A and B, burn rats with blood alcohol in the range of 240–250 mg/dl tended to have a greater degree of suppression of PP (Fig. 6A) and MLN (Fig. 6B) T cells. However, this tendency of further suppression at high blood-alcohol levels (240–250 mg/dl) was not significantly different ($P > 0.05$) compared with the suppression in T cell proliferation observed in burn animals with alcohol-exposure levels in the range of 90–100 mg/dl.

In addition to the T cell proliferative responses, we monitored bacterial numbers in MLN, spleen, and blood in animals with blood alcohol in the range of 240–250 mg/dl. As shown in Table 1, an increase in bacterial numbers in MLN of burn-injured rats with blood-alcohol levels in the range of 240–250 mg/dl compared with MLN of burn rats with blood-alcohol levels in the range of 90–100 mg/dl. However, statistic-
cal analysis of the data from these experiments did not establish a significant ($P > 0.05$) difference between the MLN of burn rats with blood-alcohol concentrations in the range of 90–100 mg/dl and in burn rats with higher blood-alcohol levels (240–250 mg/dl). Nevertheless, an interesting observation in these experiments is the consistent finding of positive cultures from blood and spleen in burn rats with blood-alcohol levels in the range of 240–250 mg/dl.

T cell IFN-γ production. In these experiments, we examined the production of IFN-γ by PP and MLN T cells. As shown in the Table 2, the levels of IFN-γ produced by PP and MLN T cells obtained from sham animals were not significantly different, regardless of their treatment. The levels of IFN-γ produced by PP and MLN T cells from burn animals gavaged with saline were significantly suppressed ($P < 0.05$) compared with the T cells from sham groups of animals. The production of PP and MLN T cells IFN-γ was further suppressed in animals receiving both alcohol and burn injury. On statistical analysis, the suppression of IFN-γ production by PP and MLN T cell of animals receiving alcohol and burn injury was found to be significantly different ($P < 0.05$) from the T cells
obtained from burn animals receiving saline and sham-injured animals.

Effect of T cell depletion on bacterial translocation. To further substantiate the role of T cells in bacterial translocation, more experiments were carried out in which we monitored bacterial presence in MLN, spleen, and blood of animals depleted of T cells. The depletion of T cells was monitored in circulation, spleen, PP, and MLN by counting CD3-positive cells using FITC-labeled antibodies to CD3 and flow cytometry.

Fig. 4. Effect of alcohol exposure before thermal injury on intestinal permeability. Intestinal permeability was determined 2 days after alcohol and burn injury by monitoring the transfer of [3H]lactulose and [14C]mannitol into blood after their infusion into intestinal segment. Values represent means ± SE from 5 animals in each group. *P < 0.05 compared with sham-injured rats regardless of their treatment; #P < 0.05 compared with burn rats receiving vehicle alone.

Fig. 5. Effect of alcohol exposure before burn injury on Peyer’s patches (PP) and MLN T cell proliferative responses. Animals were killed 2 days after alcohol and burn injury. PP and MLN were aseptically removed. Mixed PP and MLN T cells or nylon wool-purified PP and MLN T cells (5 × 10^6 cells/well) were cultured with concanavalin A (ConA; 5 µg/ml) for 66 h at 37°C, pulsed with [3H]thymidine (0.5 µCi/well), and incubated for an additional 6 h at 37°C. Incorporation of radiolabeled thymidine into cells was counted and expressed as dpm. A and B show, respectively, ConA-mediated proliferative responses of mixed PP and MLN cells. C and D represent, respectively, the proliferation of nylon-purified T cells from PP and MLN. Data are means ± SE from at least 6 animals in each group. *P < 0.05 compared with sham-injured animals regardless of alcohol exposure. **P < 0.05 compared with burn animals exposed to vehicle alone.
binding. Thus, CD8 binding sites available for additional antibody targeting on T cells, we have overcome this problem. The presence of CD3-positive cells in blood, spleen, PP, and MLN is shown in Table 3. We found that ~48% cells were CD3 positive in PP, 70% in MLN, 35% in spleen, and 54% were positive in blood of the normal rats. Intraperitoneal administration of a mixture of anti-CD4+ and anti-CD8+ antibodies in normal healthy rats resulted in the depletion of CD3+ values by 50% in PP and MLN, 80% in spleen, and 90% in blood (Table 3). As can be seen in the Table 3, such depletion of T cells caused accumulation of bacteria in MLN of healthy rats. A significant increase in bacterial numbers was observed in MLN of alcohol- and burn-injured rats, which were depleted of T cells (Table 4). Furthermore, blood cultures from the CD3-depleted animals receiving both alcohol and burn injury were positive for infection.

We selected CD3+ cells to monitor because both CD4+ and CD8+ T cells also express CD3 receptor, and thus the depletion of CD4+ and CD8+ may also result in the depletion of CD3+ cells. Furthermore, one potential problem in these experiments was that the cells obtained from animals treated with anti-CD4 and anti-CD8 antibodies may not have any anti-CD4 and anti-CD8 binding sites available for additional antibody binding. Thus flow cytometric analysis of such cells with similar anti-CD4+ and -CD8+ antibodies may not correctly represent the status of cells present. By using anti-CD3 antibody, which recognizes different receptors on T cells, we have overcome this problem. The presence of CD3-positive cells in blood, spleen, PP, and MLN is shown in Table 3. We found that ~48% cells were CD3 positive in PP, 70% in MLN, 35% in spleen, and 54% were positive in blood of the normal rats. Intraperitoneal administration of a mixture of anti-CD4+ and anti-CD8+ antibodies in normal healthy rats resulted in the depletion of CD3+ by 50% in PP and MLN, 80% in spleen, and 90% in blood (Table 3). As can be seen in the Table 3, such depletion of T cells caused accumulation of bacteria in MLN of healthy rats. A significant increase in bacterial numbers was observed in MLN of alcohol- and burn-injured rats, which were depleted of T cells (Table 4). Furthermore, blood cultures from the CD3-depleted animals receiving both alcohol and burn injury were positive for infection.

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on T cell counts in blood and in other lymphoid organs

Table 3. Effect of intraperitoneal administration of a mixture of anti-CD4 and anti-CD8 antibodies on T cell counts in blood and in other lymphoid organs

<table>
<thead>
<tr>
<th>Tissue/Organ</th>
<th>%CD3-positive cells of rats injected with:</th>
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<tbody>
<tr>
<td></td>
<td>Saline (n = 3)</td>
</tr>
<tr>
<td></td>
<td>anti-CD4 and anti-CD8 mixture (n = 3)</td>
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<tr>
<td>PP</td>
<td>48 ± 8</td>
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<tr>
<td>MLN</td>
<td>70 ± 5</td>
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<tr>
<td>Spleen</td>
<td>35 ± 5</td>
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<td>Blood</td>
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Values are means ± SE. Rats were sacrificed 2 days after injecting the mixture of anti-CD4 (OX8, 5 mg/kg) and anti-CD8 (OX38, 5 mg/kg). Blood was drawn via cardiac puncture. PP, MLN, and spleen were aseptically removed. The depletion of T cells was determined in PP, MLN, spleen, and in circulation by counting CD3-positive cells using FITC-labeled antibodies to CD3 and flow cytometry.

Table 4. Effect of alcohol exposure before burn injury on bacterial accumulation in MLN, spleen, and blood of T cell-depleted animals

<table>
<thead>
<tr>
<th>Tissue/Organ</th>
<th>T Cell Not Depleted (n = 3)</th>
<th>T Cell Depleted (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>EtOH</td>
</tr>
<tr>
<td>MLN</td>
<td>1 ± 0.5</td>
<td>2 ± 0.8</td>
</tr>
<tr>
<td>Blood</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Spleen</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE from at least 3 different animals. Rats were intraperitoneally injected with a mixture of anti-CD4 (OX8, 5 mg/kg) and anti-CD8 (OX38, 5 mg/kg) to deplete the T cells. Immediately following the administration of antibody, rats were gavaged with saline or alcohol and underwent sham or burn injury. Rats were sacrificed on day 2 postinjury. Blood was drawn via cardiac puncture. MLN and spleen were aseptically removed and homogenized. Ten microliters of blood or 10 μl of homogenates were spread on a nutrient agar plate. Plates were incubated for 24–48 h at 37°C, and the presence of bacteria was read by counting bacterial colonies appearing on the plates.

DISCUSSION

This study demonstrates that ethanol exposure before thermal injury results in a severalfold increase in bacterial translocation. Whereas no demonstrable morphological changes in the intestine were noted in any group of animals, a signifcant increase in intestinal permeability was observed in ethanol- and burn-injured rats compared with the rats receiving either injury alone. Intestinal permeability was determined by monitoring the transfer of radiolabeled lactulose and mannitol into the blood after their infusion into a segment of the intestine. Although, in this study, we have not investigated the mechanism of lactulose and mannitol transfer into the bloodstream after their infusion into the intestinal lumen, others (48, 55) have shown that lactulose passes primarily through the paracellular route, whereas mannitol (4) is transported both transcellularly across the apical membrane as well as through paracellular spaces (34, 55). Previous studies have shown that the villar tips have many more junctions of relatively smaller diameter than in the crypt region, whereas the junctions of larger diameter are present in the crypts (34, 56). The junctions of larger diameter, albeit fewer in number, may allow for the passage of both the larger lactulose as well as the smaller mannitol molecules in the crypt surface, and the smaller diameter villar junctions may only allow the smaller mannitol to permeate and thus may provide some explanation for the increased transfer of mannitol compared with lactulose in ethanol- and burn-injured rats. Nevertheless, the finding in this study, which we are unable to explain, is the increased transfer of both lactulose and mannitol from intestinal lumen into the blood in ethanol- and burn-injured rats in absence of demonstrable differences in tight junctions and paracellular spaces. However, it is likely that ethanol and burn injury may have disturbed the physiological regulation of tight junctions and paracellular spaces without causing visible damage. Such regulatory alterations in tight junctions/paracellular spaces may contribute to the enhanced permeability to lactulose and mannitol in ethanol- and burn-injured rats and, therefore, may provide some explanation for enhanced bacterial translocation in ethanol and burn injury.

Another significant factor that contributes to the increased bacterial translocation is the suppression of intestinal immune defense. Our results suggest that although there was a decrease in the proliferation in both PP and MLN T cells following burn injury alone, the suppression was maximum in the group of animals receiving both alcohol and burn injuries. The suppression of T cell proliferation is accompanied by a significant suppression of IFN-γ production by PP and MLN T cells in animals receiving alcohol and burn injury compared with the animals receiving either injury alone. Finally, we found that the depletion of CD3+ cells in healthy rats resulted in an increase in the bacterial accumulation in MLN; such depletion of CD3+ cells in burn- and alcohol-injured rats furthered the spread of bacteria to spleen and circulation. These results corroborate earlier findings from ours and other laboratories (7, 28, 54) and support the concept that the effects of acute alcohol exposure are not restricted to splenic or peripheral lymphocytes but are also

bacterial growth. Blood cultures from other groups of rats were found negative.

DISCUSSION

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present in intestinal lymphoid T cells. Although, as noted by earlier investigators (7, 28, 39), a single dose of acute alcohol exposure may not be sufficient to suppress the T cell responses; however, in combination with burn injury, the T cell response is dramatically affected. Kawakami et al. (28) suggested that acute gavage feeding of ethanol before burn injury suppressed splenic T cell mitogenesis to a greater extent than the T cells from animals with no alcohol exposure. Napolitano et al. (39) suggested that, similar to acute alcohol consumption, chronic ethanol intake also may have synergistic effects on splenic T cell proliferation following thermal injury. Suppression of T cell proliferation has been a common finding after burn, trauma, and sepsis (5, 6, 23, 29, 30, 41, 51). Similar decreases in T cell mitogenesis have been recorded after alcohol exposure (10, 24, 26, 45, 54, 56).

It is interesting to note that intestinal lymphoid organs contain more lymphocytes than the total lymphocytes from all other parts of the immune system. According to an estimate, the population of T cells found in the epithelium of the small intestine may alone account for almost 60% of all T cells in the body (38). Thus mucosal lymphocytes are the most significant part of the immune system; hence the disturbances in T cell effector functions may allow more bacteria to grow in MLN and result in their spill into blood circulation and spleen.

Whereas the mechanism underlying the intestinal T cell suppression in alcohol and burn injuries remains undefined, a number of previous studies suggested that burn injury results in hyperactivation of some cells of the immune system, leading to aberrant production of inflammatory mediators such as tumor necrosis factor-α, transforming growth factor-β, PGE₂, and corticosterone (3, 5, 12, 14, 28, 41, 51, 53). Other functions of the immune system, however, are dramatically paralyzed. These include the suppression of macrophage ability to present antigen (5, 14, 29, 36, 41, 51, 54), T cell proliferation, IL-2 production, and IL-2 receptor expression (5, 6, 23, 29). Similarly, the effects of ethanol on the immune system, independent of trauma or burn injuries, are also associated with a decrease in macrophage antigen-presenting ability, T cell proliferation, and IL-2 production (10, 24–26, 45, 56). In addition to the functional deficits identified in the cells of the immune system after alcohol or thermal injury, studies have also suggested the loss of immune cells from both systemic and intestinal lymphoid organs (22, 43). Previous studies by Szabo et al. (54) and Miller-Graziano et al. (36) suggested that the suppression of T cell function in alcohol or burn injury was secondary to macrophage dysfunction. Their studies showed T cell suppression only when T cells were cultured in the presence of macrophages. Similarly, studies by Faunce et al. (15) have implicated the role for macrophage-derived IL-6 in T cell suppression in ethanol- and burn-injured mice. In contrast, previous studies including our own (5, 6, 23, 29) as well as the data presented here suggest a suppression of T cell mitogenesis both in the absence or presence of macrophages or other adherent cells. Furthermore, we have shown attenuated anti-CD3-linked signaling molecules in freshly isolated T cells (5, 8, 9). The differences between our studies and others, however, are likely due to factors such as the degree of burn or route of alcohol administration. The studies by Faunce et al. (15) have used a mouse model of 15% burn and intraperitoneal alcohol injection, whereas in our studies, rats were orally gavaged with alcohol and received a 30% burn.

The intestinal immune system is the first line of defense against enteric bacteria. The lymphoid tissues associated with the intestine are exposed continuously to antigens in the lumen of gut. Under healthy conditions, a few indigenous bacteria are known to continuously translocate to MLN, but because of intact immune defense, these bacteria do not survive. Thus MLN from normal animals remains relatively sterile. However, injuries, such as alcohol and burn, disrupt the effective mucosal defense to intraluminal microorganisms, leading to the passage of viable bacteria across the luminal barrier to MLN and distant organs. The definitive pathways by which bacteria reach MLN and systemic organs are uncertain. Macrophages may transport bacteria from the gut to the MLN, and from the MLN they can enter systemic circulation. The fact that appropriate activation of intestinal T cells is critical in maintaining immunity against the translocation of enteric bacteria is derived from many sources (19–21, 31, 42, 44, 52). Owens and Berg (42) noted spontaneous gut bacterial translocation to MLN, spleen, and liver in athymic (nu/nu) mice, whereas no translocation was noticed in heterozygous (nu/+ ) or nude (+/+ ) mice grafted with thymus. Yet another study showed that depletion of CD4⁺ and CD8⁺ T cells also resulted in increased translocation of Salmonella typhimurium and other enteric bacteria (31, 37, 44). Additional studies showed the ability of adoptively transferred T cells to confer protection against a number of bacterial infections, including Escherichia coli (19, 20), Mycobacterium leprae (18), Listeria monocytogenes (47), S. typhimurium (37), or Bordetella pertussis (32).

We found a significant suppression in intestinal T cell IFN-γ production. Such a decrease in IFN-γ may affect the phagocytic ability of macrophages and phagocytic cells and thus allow bacteria to multiply and transfer to extraintestinal sites. IFN-γ produced by the intestinal T cell helps in resolution of Yersinia enterocolitica infection (30). In another study (2), IFN-γ was shown to confer protection against S. typhimurium invasion of epithelial cells and fibroblasts. Moreover, the decrease in IFN-γ enhanced the susceptibility to infection. IFN-γ-deficient mice showed impaired ability of macrophages to produce nitric oxide and superoxide anion necessary to kill bacteria (21, 43). Other cytokines or chemokines produced by T cells help in maintaining humoral immunity such as T cell-dependent antibody production, recruiting, and activating microbicidal activities of cells such as macrophages and neutrophils (31, 52). Cytolytic effector functions, as expressed by CD8⁺ T cells, are also mediated by either direct cell-to-cell contact or by secreting cytokines (21). These studies together suggest that...
alterations in IFN-γ or in other T cell effector responses will potentially result in perturbation of the complex network of host defense, resulting in immune dysfunction.

In summary, the results presented here suggest that enhanced intestinal permeability in rats receiving alcohol and burn injury may cause an increase in bacterial translocation, whereas the suppression in intestinal T cell effector responses, such as the decrease in the production of IFN-γ, may further their accumulation and spread to extraintestinal sites.

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