The Degree of Bacterial Translocation is a Determinant Factor for Mortality After Burn Injury and is Improved by Prostaglandin Analogs

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Bacterial translocation and related mortality rates were examined in previously transfused BALB/c mice that were gavaged with ^{14}C radioisotope-labeled *Escherichia coli* before inflicting a 20% full-thickness flame burn. Radionuclide counts were measured in blood obtained by retro-orbital puncture 4 hours postburn, and survival was recorded for 10 days. Radionuclide counts in the blood correlated well with both radionuclide counts and numbers of viable bacterial in the tissues. Survivors had significantly less bacterial translocation as evidenced by blood radionuclide counts compared with nonsurvivors, and there was a significant inverse correlation between the degree of translocation and the length of survival. In the next experiment, the prostaglandin E (PGE) analogs misoprostol, enisoprost, or 16,16-dimethyl PGE_2 were administered to transfused animals for 3 days before burn. Prostaglandin E analogs significantly reduced bacterial translocation as measured by blood radionuclide counts 4 hours postburn and improved survival. The data demonstrate that the intensity of bacterial translocation after burn injury is significantly associated with subsequent death. Improvement of survival by PGE analogs is associated with decreased bacterial translocation.

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THE PASSAGE OF large particles through the intestinal barrier, initially called persorption, was recognized as early as 1844 by Herbst.[1] Volkheimer and Schulz[2] reported in 1968 that intact starch particles could be transported across the intestine of normal human subjects. In 1969, Krause et al.[3] reported a bold clinical experiment showing that *Candida albicans,* when taken by mouth, would cross the intestinal barrier in a normal human and cause transient fungemia and clinical illness. Berg and Garlington[4] introduced the term "bacterial translocation" in 1979 to mean the passage of viable enteric bacteria through the intact gastrointestinal tract to the mesenteric lymph nodes (MLN) and beyond. Subsequently, the term "microbial translocation" was introduced to more accurately reflect the passage of both viable and dead microbes as well as their products through the intact mucosal barrier.[5] Several investigators stressing this process have demonstrated an increased incidence of bacterial translocation associated with hemorrhagic shock,[6] thermal injury,[7,8] intestinal obstruction,[9,10] endotoxemia,[11] intravenous hyperalimentation,[12] and antibiotic therapy.[13] Bacterial translocation has been postulated to be a potential mechanism of systemic infection,[14,15] postinjury hypermetabolism,[16] sepsis,[17] and multiple system organ failure.[18,19] Although a few studies provide indirect evidence that microbial translocation may be a major initiating pathophysiologic event for subsequent complications in the postinjury period,[14,15,18,19] direct evidence for this has not been well documented. If bacterial translocation were proven to be a causative factor in adverse outcome rather than a passive associated event, prevention of this process could become an important therapeutic tool for the management of critically ill patients.

The purpose of this investigation was to assess the potential relationship between the degree of bacterial translocation and outcome (survival) after burn injury in the same individuals. In addition, the effects of prevention of bacterial translocation on outcome was studied by using therapeutic interventions to improve gut mucosal barrier function. Prostaglandin E (PGE) analogs were used because they have been shown to have protective effects on the gastrointestinal mucosa[20] and to prevent bacterial translocation.[21]

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Materials and Methods

Animals and Animal Care

Adult female BALB/c mice (H-2d) weighing 19 to 21 g (Charles River Laboratory, Wilmington, MA) and adult female C3H/HeJ mice (H-2k) 20 to 25 g (Jackson Laboratory, Bar Harbor, ME) were caged in groups of five, and provided food (Rodent Laboratory Chow 5001, Purina Mills, Inc., St. Louis, MO) and water *ad libitum* during a quarantine period of at least 1 week so they could adapt to the standard laboratory environment, and to detect any pre-existing diseases. The protocols were approved by the University of Cincinnati Medical Center's Institutional Animal Care Use Committee, and the animals were housed in an AAALAC-approved facility. All investigations adhered to the Guide for the Care and Use of Laboratory Animals as set forth by the Committee on the Care and Use of Laboratory Animals, National Research Council, the United States Department of Health and Human Services and the National Institutes of Health.

Blood Transfusion

Under methoxyflurane anesthesia, C3H/HeJ mice were bled and killed by cardiac puncture. The harvested blood was mixed at a 4:1 volume ratio with anticoagulant citrate phosphate dextrose adenine solution UPS (Fenwal Laboratories, Deerfield, IL) and stored at 4 C overnight. Five days before burn injury, BALB/c mice were transfused through a tail vein with 0.2 mL C3H/HeJ blood and housed with food and water *ad libitum.*

Treatment With Misoprostol, Enisoprost, or 16,16-Dimethyl PGE_2

Misoprostol and enisoprost (Searle Research and Development Co., Skokie, IL) were supplied in powder form. Each drug was suspended in sterile distilled water and further diluted to obtain final concentrations of 40 μg/mL. 16,16-Dimethyl PGE_2 (Cayman Chemical Co., Ann Arbor, MI) was dissolved in ethanol to a final concentration of 16 μg/mL. These suspensions were made daily before usage and stored briefly at 4 C until administration. The preparations were given by gavage (200 μg/kg/day misoprostol and enisoprost) or intraperitoneally (80 mg/kg/day 16,16-dimethyl PGE_2) once a day for three days before burn. Preliminary experiments showed that 16,16-dimethyl PGE_2 was not effective when given orally.

Preparation of Bacteria

Escherichia coli (53104) was inoculated into glucose-free minimal nutrient media, and ^{14}C glucose 500 μCi (New England Nuclear, Boston, MA) was added as described previously.[22] The suspension was incubated overnight at 37 C. The culture then was centrifuged to pellet the organisms, washed twice, quantitated with a Klett densitometer, and adjusted to a concentration of 10^{10} viable bacteria/0.1 mL as confirmed by quantitative culture. Radionuclide counts were made of the diluted suspension.

Gavage and Burn Procedures

One day before burn injury, the animals had hair of the torso removed by clipping. Food was withheld for 18 hours, but water was provided *ad libitum* before gavage with 0.1 mL of the translocation probe (10^{10} ^{14}C *E. coli*) while the animals were awake. After gavage, they were anesthetized with methoxyflurane inhalation, and a 20% full-thickness flame burn was inflicted using the technique of Stieritz and Holder.[23] Saline, 0.5 mL, was given intraperitoneally immediately after burn injury for fluid resuscitation, and the animals were allowed to recover from anesthesia with free access to food and water.

Experimental Design

Experiment I. This preliminary experiment was done to establish the validity of evaluating the extent of bacterial translocation by the measurement of radionuclide counts in the blood.

Twenty-seven BALB/c mice, either treated with misoprostol or enisoprost or nontreated (n = 9 each), were gavaged with ^{14}C *E. coli,* burned, and killed at 4 hours after burn. The animals were prepared with 70% alcohol, and aseptic techniques were used to remove the MLN and liver. Blood also was withdrawn by cardiac puncture. The tissues obtained were individually weighed, homogenized with 1 mL sterile saline, and 100 μL of the homogenate was plated (diluted when appropriate) on brain heart infusion agar plates (Baltimore Biological Laboratories, Baltimore, MD) for quantitative bacterial colony counts after 18 hours of aerobic incubation at 37 C. The remainder of the homogenates were lyophilized, decolorized when needed, and disintegrations per minute (dpm) of ^{14}C were determined by liquid scintillation counting (Beckman Model LS 3133 liquid scintillation counter, Beckman Instruments Inc., Fullerton, CA) as previously described.[22] Blood was similarly processed through the decolorization procedure.

The viable colony counts and the radioactivity in tissues (dpm) were calculated and adjusted to be expressed as bacteria/gram or milliliter of tissue or dpm/gram or milliliter of tissue, as previously described.[22]

Experiment II. Thirty blood-transfused BALB/c mice were gavaged with ^{14}C *E. coli* and burned. Four hours later, animals were reanesthetized to withdraw 80 μL of blood by retro-orbital puncture and then observed for 10 days for survival. During the entire postburn period, an-

imals had free access to water and food. The blood radionuclide count was determined as described above.

Experiment III. Misoprostol (n = 20), enisoprost (n = 20), or 16,16-dimethyl PGE_2 (n = 15) were administered to transfused animals for 3 days before burn, and the same observations as in experiment II were made. Control animals (n = 20) were similarly gavaged with distilled water.

Statistics

Data are expressed as mean ± standard error of mean. A standard one-way analysis of variance followed by Duncan's new multiple range tests were used to compare means. Survival rates were analyzed by chi square test. Linear regression analysis was also used.

Results

Experiment I

Figure 1 shows the relationship between radionuclide count (dpm) in the blood and tissues (MLN and liver). Significant positive linear correlations were noted (*versus* MLN, p = 0.0001, r = 0.855; *versus* liver, p = 0.0001, r = 0.744). There were also significant positive correlations between blood dpm and viable colony counts in the MLN (p = 0.0013, r = 0.587) or liver (p = 0.0001, r = 0.859) (Fig. 2). In this experiment, a threshold of approximately 250 dpm/mL blood appeared to be necessary for bacteria to be cultured from the tissue (*e.g.*, above 250 dpm/mL blood, p = 0.008, r = 0.879 for colony-forming units (CFU)/g liver).

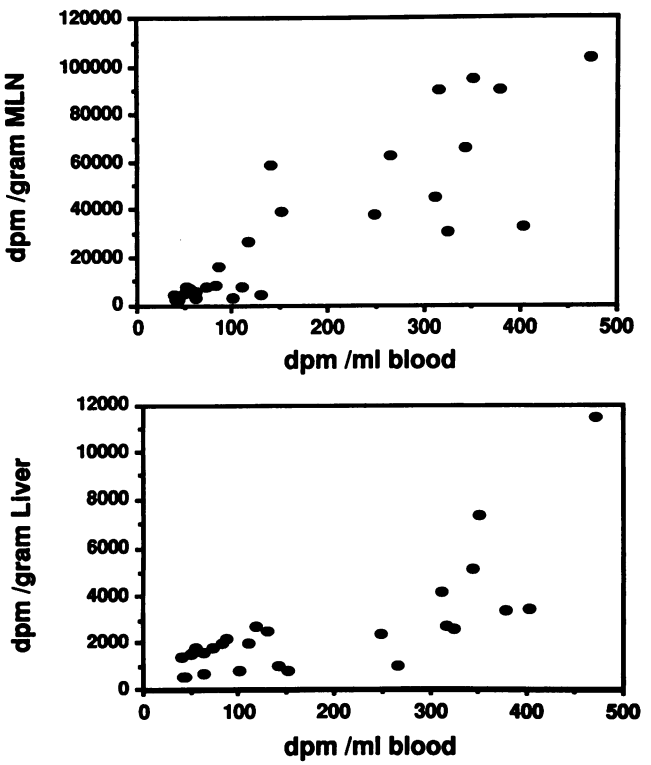

FIG. 1. Correlation of disintegrations per minute count per milliliter of blood and disintegrations per minute count per gram of liver or MLN 4 hours after gavage and burn (n = 27). A statistically significant correlation was seen: p = 0.0001, r = 0.855 *versus* MLN; p = 0.0001, r = 0.744 *versus* liver.

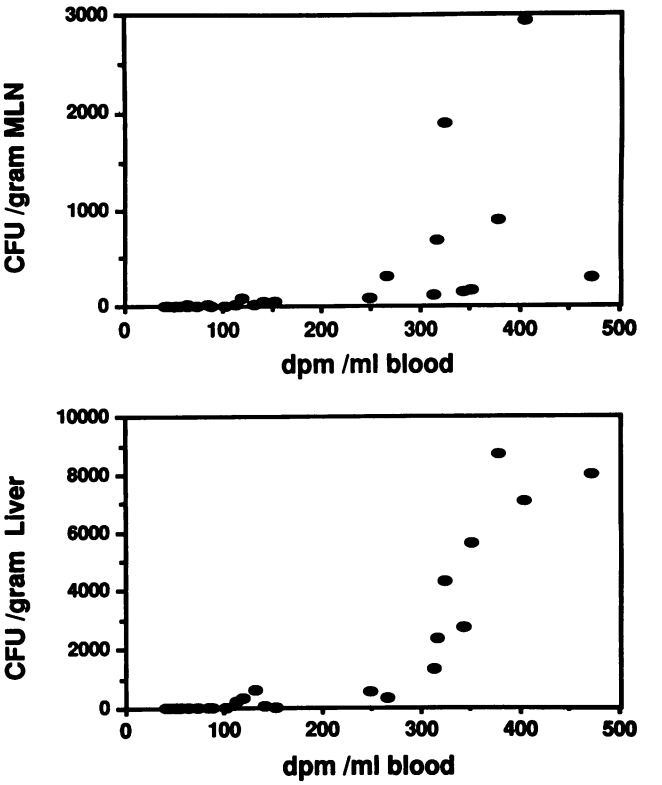

FIG. 2. Correlation of disintegrations per minute count per milliliter of blood and CFU count per gram of liver or MLN 4 hours after gavage and burn (n = 27). Bacterial killing in both MLN and liver was efficient to a threshold of about 250 dpm/mL blood. A statistically significant correlation was seen: p = 0.0013, r = 0.587 *versus* MLN; p = 0.0001, r = 0.859 *versus* liver.

Experiment II

The overall survival rate during the 10-day study period in experiment II was 20%. The magnitude of bacterial translocation in survivors 4 hours after gavage and burn, as assessed by radionuclide counts in the blood, was 263 ± 19 dpm/mL. The value was significantly lower than in nonsurvivors, 351 ± 15 dpm/mL ($p < 0.05$) (Fig. 3). Figure 4 shows the relationship between the blood radionuclide counts (degree of bacterial translocation) 4 hours after burn and the length of survival after burn injury. In the nonsurviving animals, there was a significant inverse correlation between bacterial translocation (dpm) and the length of survival ($p < 0.05$, r = 0.415).

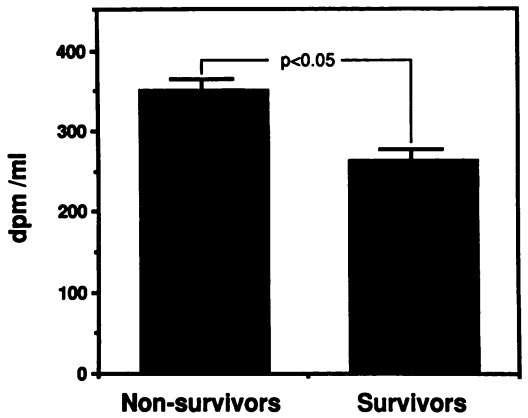

FIG. 3. Radionuclide counts in the blood 4 hours after gavage with 10^{10} ^{14}C *E. coli* and burn in experiment II. Data are expressed as disintegrations per minute per milliliter of blood. Survivors had significantly lower disintegrations per minute counts compared with nonsurvivors. Values are mean ± SEM.

Experiment III

Figure 5 shows the survival curves for misoprostol, enisoprost, and 16,16-dimethyl PGE_2-treated groups and nontreated controls. Prostaglandin E analog treatment significantly improved the survival after burn injury. The best survival was obtained by misoprostol administration (70%, $p < 0.001$ *versus* 10% for nontreated group; $p < 0.05$ *versus* 40% for 16,16-dimethyl PGE_2 group), followed by enisoprost (60%, $p < 0.01$ *versus* nontreated group) and 16,16-dimethyl PGE_2 (40%, $p < 0.05$ *versus* nontreated group). The magnitude of bacterial translocation measured by blood dpm counts 4 hours after burn injury for these groups are shown in Figure 6. All three PGE analogs significantly reduced the degree of bacterial translocation as measured by blood dpm counts. Translocation was least in the enisoprost group, but this was not significant compared with groups treated with the other two PGE analogs.

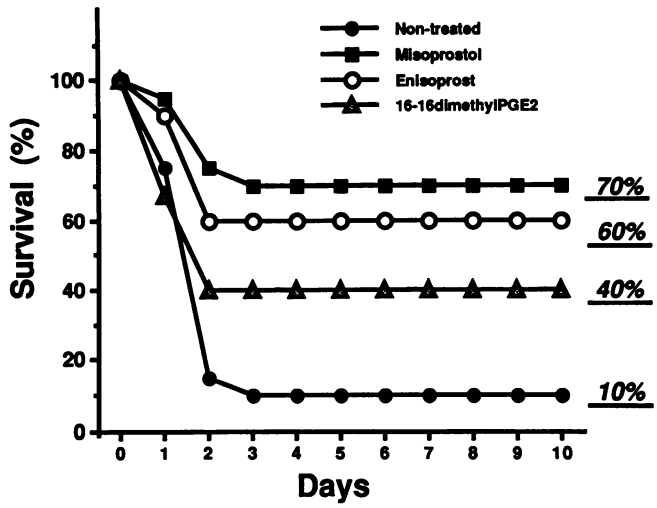

FIG. 5. Survival rate of transfused, gavaged, and burned mice treated for 3 days with misoprostol (200 μg/kg/day), enisoprost (200 mg/kg/day), or 16,16-dimethyl PGE_2 (80 mg/kg/day). Misoprostol *versus* nontreated, $p < 0.01$; misoprostol *versus* 16, 16-dimethyl PGE_2, $p < 0.05$; enisoprost *versus* nontreated, $p < 0.01$; 16,16-dimethyl PGE_2 *versus* nontreated, $p < 0.05$ (chi-square test).

Discussion

In the last decade, microbial translocation has received increasing recognition as one of the potential causes of

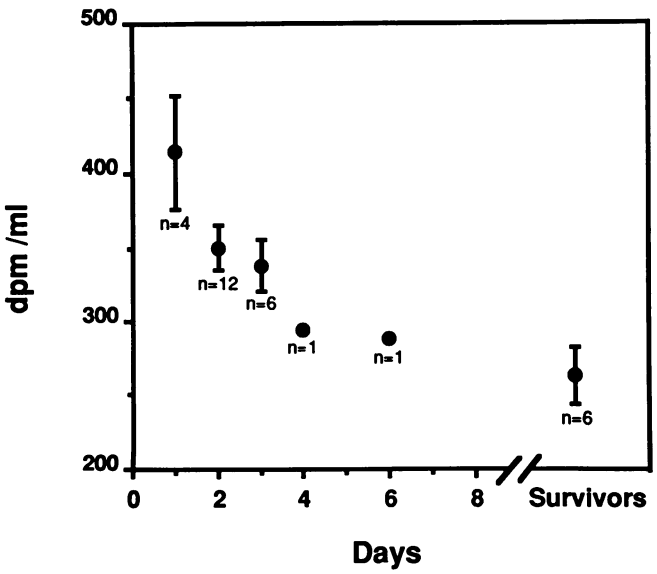

FIG. 4. Correlation between the degree of translocation as measured by radionuclide count (dpm/mL) in the blood and survival time (days) after burn in experiment II. A statistically significant correlation was seen: $p < 0.05$, $r = 0.415$, $n = 24$. Values are mean ± SEM.

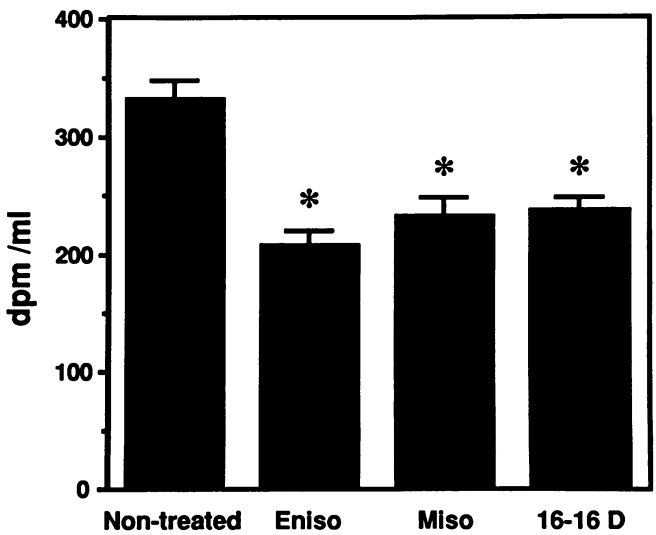

FIG. 6. Radionuclide counts in the blood 4 hours after gavage with 10^{10} ^{14}C *E. coli* and burn in experiment III. Data are expressed as disintegrations per minute per milliliter of blood. Values are mean ± SEM. *p < 0.05 *versus* nontreated group.

systemic infections,[14,15] postinjury hypermetabolism,[16] multiple-system organ failure in seriously ill patients[18,19] and clinical sepsis with an undefined focus of infection.[17] Evidence that the extent of bacterial translocation directly affects the outcome of various diseases, however, is sparse. The current study was designed to establish the relationship between the degree of bacterial translocation and survival after burn injury in individual animals and to test the hypothesis that administration of PGE analogs (misoprostol, enisoprost, and 16,16-dimethyl PGE_2) could improve gut barrier function. The results indicate that overall survival and length of survival after burn injury were highly associated with the extent of bacterial translocation measured 4 hours postburn by blood radionuclide counts in animals gavaged with ^{14}C *E. coli.* Prostaglandin E analogs significantly reduced the amount of bacteria that translocated through the gut and was associated with improved survival.

Bacterial translocation can be evaluated in several ways. The most common and widely used method is to recover the translocated viable microorganisms in tissues such as mesenteric lymph nodes, but this does not precisely reflect the degree of translocation because most bacteria that translocate are killed rapidly by the host and are detected. We have recently developed a model for evaluating bacterial translocation not only by recovery of viable bacteria in various tissues but also by measurement of radionuclide counts using viable ^{14}C radioisotope-labeled microorganisms introduced into the gastrointestinal tract by gavage.[22] Using this method, as was confirmed in experiment I, a small but variable amount of radioactivity is detectable in the blood that is proportional to the amount of radioactivity in various tissues, thus allowing blood sampling to monitor the relative extent of total translocation from the intestine using the ^{14}C *E. coli* probe. It is interesting that below a critical point of 250 dpm/mL blood, detectable CFU were found infrequently in MLN and liver. Evaluation of bacterial translocation in subsequent experiments was done exclusively by measuring radionuclide counts in the blood, enabling observation of the degree of bacterial translocation without killing the animals and direct correlation with mortality.

It has been postulated that blood transfusions lead to immunosuppression and an increase in infections.[24-27] We have developed a blood transfusion/burn model with a gavage of 10^{10} *E. coli* that is associated with a mortality rate of approximately 80% to 90% during 10 days of observation.[28] These studies have shown also that blood transfusion itself did not affect the number of bacteria that pass through the gut mucosa, but significantly impaired the killing of bacteria that did translocate and reduced the numbers of animals surviving postburn.[28] Because blood transfusions are used commonly in burn injury, trauma, and surgical patients, this model may reflect well the pathophysiologic perturbations of critically injured patients. The high mortality rate in this model is also ideal for the evaluation of therapeutic interventions.

The reduction of bacterial translocation by administration of PGE analogs observed in the current study was consistent with a previous investigation from our laboratory that showed that the administration of 200 μg/kg/day of misoprostol or enisoprost significantly reduced the amount of *E. coli* translocation to MLN, liver, and spleen 4 and 24 hours postburn.[21] The current study further showed that PGE treatment significantly improved survival after burn and that the extent of translocation in treated animals was related to both incidence and length of survival. Several investigators have reported recently that PGE was effective in hemorrhagic shock,[29] traumatic shock,[30] or adult respiratory distress syndrome (ARDS).[31] Although the authors did not focus their attention on bacterial translocation in these models, it is possible that deceased bacterial translocation caused by PGE contributed to the improved outcome.

Prostaglandin Es have been reported to have the property of "cytoprotection," the ability to prevent gastrointestinal mucosal damage from various necrotizing agents such as alcohol, HCl, NaOH, or hypertonic NaCl.[20] Cytoprotection has been attributed to various effects of PGs, for example, increased gastrointestinal blood flow, enhancement of mucus secretion, promotion of bicarbonate secretion, and cyclic AMP production.[32] Because the event of bacterial translocation seems to be associated with disorders that are likely to disturb splanchnic blood flow,[33,34] this property of PGE to maintain mucosal flow may play an essential role in preventing bacterial translocation. In addition, enhanced secretion of mucus, which will provide a viscous physical barrier between the bacteria and gastrointestinal luminal cells, suggests another important mechanism for the decreased translocation by PGE analogs seen in the current study. Prostaglandin Es are also known to downregulate the synthesis of tumor necrosis factor (TNF) *in vitro.*[35-37] Recently, Mahatma et al.[38] reported that misoprostol prevented endotoxin-induced gastric mucosal injury. They also reported that endotoxin-stimulated TNF production was decreased *in vivo* by PGE_1. Tumor necrosis factor is thought to be a proximal mediator of endotoxin-associated tissue injury and to lead to gastrointestinal mucosal damage.[39] Although bacterial translocation can occur in intact gastrointestinal mucosa, it is possible that TNF played a role in the process of bacterial translocation. It is also known that endotoxemia itself promotes bacterial translocation.[11] The exact mechanisms for PGE-related prevention of bacterial translocation in the current study, however, is not clear.

Although a significantly lower survival rate was observed in the group treated with 16,16-dimethyl PGE_2 compared with the misoprostol group, there was no dif-

ference between the degree of bacterial translocation among three different groups treated with PGE analogs. The immunosuppressive effects of PGE_2 are well known and have been reported earlier.[40,41] Prostaglandin E_1 analogs also have been implicated as having an immunosuppressive effect in transplantation models,[42,43] but prospective clinical studies using enisoprost have failed to demonstrate a beneficial effect in renal transplantation.[44] It also was reported that oral misoprostol treatment did not improve allograft survival in an animal model.[45] Data from our laboratory further showed that both misoprostol and enisoprost (using the same dose as used in this study) had little inhibition of clearance of translocated bacteria in the burned mouse model.[21] Thus, different immunosuppressive effects may be one of the explanations for the different survival among groups treated with PGE_1 or PGE_2 analogs.

In summary, our data demonstrate that the degree of microbial translocation from the intestine is highly associated with both extent and length of survival after burn injury. Survival was improved by the administration of PGE analogs, which improved gut barrier function. The data support the hypothesis that bacterial translocation can be a determinant factor for outcome in critical illness. Prevention of bacterial translocation by drugs such as PGE_1 analogs could be an important therapeutic approach to lessen the morbidity and mortality rates in critically ill patients.

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Discussion

Dr. Edwin Deitch (Shreveport, Louisiana): I would like to compliment Dr. Fukushima on an excellent presentation. Conceptually, the strength of this study is its basic attempt to clarify the clinical significance of bacterial translocation, and its direct effect on mortality rate.

From our own studies, we would suggest that under the right conditions bacterial translocation can produce a lethal septic syndrome. This slide illustrates this concept using our zymosan translocation model. Zymosan, which is a major component of the cell wall of yeast, causes a systemic inflammatory state that we have shown to induce bacterial translocation and death in a dose-dependent fashion. To determine whether death is directly related to loss of gut barrier failure and subsequent bacterial translocation, we treated these animals with antibiotics. What we found was that, at a zymosan dose of 1 mg/g body weight, the lethal effects of zymosan appeared to be directly related to gut-origin sepsis, because cefoxitin reduced the 7-day mortality rate from 100% to 20% (J Trauma 1992; 32:141–147). At a higher dose of zymosan (2 mg/g), antibiotics did not improve survival. These results indicate that bacterial translocation can directly increase mortality rate, but if the magnitude of the insult is sufficiently severe then loss of intestinal barrier function and bacterial translocation becomes of limited causal importance in the development of multiple organ failure or death.

Because the authors found that the prostaglandin analogs improved survival, my questions center around how the prostaglandin analogs may have been beneficial.

We know that prostaglandins have multiple physiologic effects, including a mucosal cytoprotective effect, and the ability to downregulate cytokine activity and to modulate the immune system. Consequently, I wonder if you have any data on mucosal histology or permeability that would shed light on the mucosal cytoprotective arm of prostaglandin activity. We as well as others have shown that after thermal injury in the BALB/c mice the mucosa is damaged and the permeability is increased. Because prostaglandin E_1 (PGE_1) is clearly cytoprotective, could this beneficial effect on translocation be because the mucosal barrier is being preserved?

Second, most of your animals die within 48 hours of injury. This is very consistent with either a rapid septicemia or massive cytokinemia. Because the prostaglandins may have an effect on cytokines, I wonder if you had measured cytokine levels in your animals to investigate the relative effects of cytokines *versus* bacteria on survival.

Last, I would like to ask you a question about the role of prostaglandin E_2. Prostaglandin E_2 is generally considered to be immunosuppressive; thus it is interesting that in fact it improved survival. Because PGE_2 has a number of other effects as well, I wonder if you have looked at quantitative levels of viable bacteria to see if there is any relationship between outcome and the number of live bacteria rather than radioactivity? I think this measurement is critically important, because you have beautifully shown before that the level of radioactivity does not reflect the number of live organisms, and it may be that the number of live organisms is a prime determinant of survival.

Dr. David N. Herndon (Galveston, Texas): This excellently presented paper adds considerably to the wealth of data that Dr. Alexander's group has contributed in the area of bacterial translocation.

The technique described here after gavage of radioactively labeled *Escherichia coli* is a major step forward because it allows survival studies as opposed to postmortem analyses in previous studies. I really think this is a major contribution. I do have several questions. We have shown that administration of thromboxane synthetase inhibitors in the 40% burned pig model restores postburn mesenteric vasoconstriction and attenuates bacterial translocation. My question is whether you think prostaglandin E (PGE) works by improving gut mucosal blood flow or by other mechanisms alluded to by Dr. Deitch improving structural integrity of the gut mucosa? Are there any studies you have to address that question?

The other questions are minor. Pretreatment of burn is not practical and pretransfusion of burn patients is not realistic. Have you designed any post-treatment experiments with PGE? If it does indeed work on mesenteric vasoconstriction, perhaps subsequent endotoxin challenges after burn injury could be attenuated by the use of this substance. Post-transfusion treatment burns would be more realistic rather than pre-transfusion treatment of the burned animals. Are any experiments designed in that direction?

Dr. Frank G. Moody (Houston, Texas): We have been interested in the role of the gut in multiple organ failure and have studied how bacteria might get out of its lumen to cause the sequence of events that occur. We have studied two models.

One is a bile-duct–ligated rat that causes pancreatitis that creates a retroperitoneal burn. And curiously this creates an ileus. There is a decrease in propulsion, an overgrowth of bacteria, and a movement of bacteria into the mesenteric lymph nodes under these conditions. But by day 3 this all disappears. The pancreatitis progresses, but the gut motility comes back, the bacterial counts go down, and the translocation disappears. So there is a very, very tight relationship between the number of bacteria in the gut and their movement out of the gut and into the mesenteric lymph nodes.

Now, if you want to get bugs to go upstream in the portal system of a rat, put them on morphine. Each day they must receive more morphine, because they become used to the morphine very quickly. Also they must receive total parenteral nutrition. Within a period of 5 days under these conditions bacteria translocate to all of the splanchnic organs. It is a great translocating model.

So my question relates these observations, because in your experiments you insult the mouse and you gavage material into its stomach. But you also starved the mouse for 18 hours. So what effect did that have on this