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• BASIC RESEARCH •

Risk factors of development of gut-derived bacterial translocation in thermally injured rats

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Abstract

AIM: Studies have demonstrated that gut-derived bacterial translocation (BT) might play a role in the occurrence of sepsis and multiple organ dysfunction syndrome (MODS). Yet, no convincing overall analysis of risk factors for BT has been reported. The purpose of this study was to evaluate the related factors for the development of BT in burned rats.

METHODS: Wistar rats were subjected to 30% third-degree burns. Then samples were taken on postburn d 1, 3, and 5. Incidence of BT and counts of mucosal bifidobacteria, fungi and *E. coli*, mucus sIgA, degree of injury to ileal mucosa, and plasma interleukin-6 were observed. Univariate analysis and multivariate logistic regression analysis were performed.

RESULTS: The overall BT rate was 53.9% (69 in 128). The result of univariate analysis showed that the levels of plasma endotoxin and interleukin-6, the counts of mucosal fungi and *E. coli*, and the scores of ileum lesion were markedly increased in animals with BT compared with those without (P=0.000-0.005), while the levels of mucus sIgA and the counts of mucosal bifidobacteria were significantly reduced in animals with translocation compared with those without (P=0.000). There was a significant positive correlation between mucus sIgA and the counts of mucosal bifidobacteria (r=0.74, P=0.001). Moreover, there were strong negative correlations between scores of ileum-lesion and counts of bifidobacteria (r=-0.67, P=0.001). Multivariate logistic regression revealed that ileum lesion score (odds ratio [*OR*] 45.52, 95% confidence interval [*CI*] 5.25-394.80), and counts of mucosal bifidobacteria (*OR* 0.039, 95% *CI* 0.0032-0.48) were independent predictors of BT secondary to severe burns.

CONCLUSION: Ileal lesion score and counts of mucosal bifidobacteria can be chosen as independent prognosis factors of the development of BT. Specific interventions targeting these high-risk factors might be implemented to attenuate BT, including strategies for repair of damaged intestinal mucosae and restoration of the balance of gastrointestinal flora.

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INTRODUCTION

Sepsis and multiple organ dysfunction syndrome (MODS) remain the leading causes of death 72 h after a severe burn and other traumas. Early MODS after a severe injury is usually due to an excessive and overwhelming malignant systemic inflammatory response and massive hemorrhagic shock as a result of the initial insults. On account of the finding that as many as 30% patients died of early sepsis and MODS with no identifiable septic foci[1], some investigators postulated that systemic infections might be originated from the gut.

At present, many animal and a few clinical studies have demonstrated that gut-derived bacterial translocation does play a role in the occurrence of early sepsis and MODS[2-5]. Because most of the pathogens come from the gut, the gastrointestinal tract is even termed as an "undrained abscess" under certain circumstances such as trauma, endotoxemia, hemorrhage, and thermal injury. A generally accepted theory is that translocation of luminal bacteria and toxins is mechanically linked to the following factors, namely disruption of the normal balance in indigenous microflora with subsequent overgrowth of potentially pathogenic bacteria, impaired intestinal immunologic barrier of the host, and disruption of the mucosal physical barrier of the gut[1]. However, to our knowledge, what role these three factors play in the incidence of bacterial translocation under some conditions has not been fully elucidated.

In the past decade, literature on the mechanisms and methods of treatment and prevention of bacterial translocation has been substantial. However, to date no convincing overall analysis of risk factors for bacterial translocation has been reported.

The purpose of our study was to clarify the individual role of intestinal mucosa, mucosal flora, and gut sIgA in producing bacterial translocation in scalded rats, in an attempt to propose a formula for predicting the probability of incidence of bacterial translocation by using univariate analysis and multivariate logistic regression.

MATERIALS AND METHODS

Animals

Wistar rats, weighing 180 to 220 g, male and female in equal number, were purchased from the Experiment Animal Center of Third Military Medical University in Chongqing, China. Animals were housed in separate steel cages in a temperature-controlled room with a 12-h light-dark cycle, and acclimatized for at least seven days prior to use. Animals had free access to an irradiated commercial rodent diet and autoclaved water *ad lib*. All experimental manipulations were undertaken in accordance with the NIH Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of the Institute of Burn Research, Southwestern Hospital, Third Military Medical University, Chongqing, China.

Burn injury

After an overnight fast, rats were weighed, numbered, and anesthetized with sodium pentobarbital (40 mg/kg body mass, i.p.), with the dorsal hair shaved, and then subjected to 30% total body surface area skin full-thickness thermal injury, which was produced by exposure to 94 ℃ water bath for 18 s using a wood template with an aluminium wand for fixing rat abdomen. Rats were quickly dried and resuscitated with Ringer' s lactate (40 mL/kg, i.p.) immediately after injury. Animals were then allowed to fully recover from anesthesia before being returned to their cages, and had free access to radiated commercial rodent chow and autoclaved water *ad lib*. Sulphadiazine silver suspension (20 g in 100 mL water) was applied to the wounds once a day to prevent infection. Samples were taken on post-burn d 1, 3, and 5. Fifty animals were included at each time point except for sham burned group with ten.

Microbiologic analysis

Techniques for culturing and bacterial translocation studies were performed with a modification of the methodology described by Tadros *et al*[6]. After the animals were anesthetized (50 mg/kg body mass, i.p.), their abdomens were shaved, sterilized with tincture of iodine and 750 mL/L alcohol, and opened through a midline incision with sterile scissors. Under aseptic conditions, blood was obtained from portal vein and vena cava under direct visualization, and a swab culture was taken from the exposed belly cavity. Then mesenteric lymph nodes (MLN), spleen, liver, and kidney were obtained and weighed. Each organ was homogenized in brain heart infusion broth. Two hundred μL homogenates from tissues, as well as 200 μL blood, were inoculated on both Gram-negative bacteria-specific MacConkey' s agar and blood agar. A duplicate culture was made for each specimen. All specimens were incubated at 37 ℃ for 24 h. Positive specimens were sub-cultured, and the bacteria were identified by standard bacteriologic techniques. Cultures were considered positive when more then 100 colonies per gram of tissue were found. No culture for obligate anaerobics was made, because they had a low tendency to translocate to extra-intestinal sites.

Following removal of the aforementioned organs, the terminal ileal loop was excised, opened longitudinally, then its content was wiped off lightly with sterile cotton swabs, rinsed three times with 10 mL sterile 0.01 mol/L phosphate-buffered saline (PBS). The residual liquid was dried with sterile filter paper. The specimen was then put into a CO_2 filled bottle immediately. The samples were weighed, homogenized with a sterile blender, and diluted by 10 -fold with brain heart infusion broth. One hundred μL desired diluted specimen was poured separately onto the *E. coli*-specific MacConkey' s agar, bifidobacteria-specific BLB agar, and fungi-specific medium (modified by including Imipenium 30 μg/mL). The cultures were all duplicated. Plates were incubated for 24 h for aerobic bacterial culture, and 72 h for anaerobic and fungus cultures, at 37 ℃, respectively. Then colonies of bacteria or fungi were counted, and the suspiciousness was identified using standard microbiologic technique. All plates and brain-heart infusion were purchased from Shanghai Med&Chem Institute, Shanghai, China.

Quantitative culture results were expressed as the number of $\log_{10}$ colony-forming units (CFU) per gram tissue. The terminal ileal loop was used because bacterial translocation correlated with colonization of the ileum rather than that of the colon[6]. The limit detection of the assay was 10 bacteria.

Plasma endotoxin measurement

Portal blood was collected and put into pyrogen-free polypropylene tubes containing 2 μL of sterile heparin. Platelet-rich plasma was obtained by centrifugation at 4 ℃, 260 r/min for 10 min, and then aliquots of which were prepared and transferred to sterile pyrogen-free tubes under laminar air flow, and stored at -35 ℃ until use. Plasma endotoxin concentration was measured by the chromogenic limulus amebocyte lysate (LAL) method. The procedure followed was based on the protocol provided with the kit (Shanghai Med&Chem Institute, Shanghai, China). Briefly, in order to avoid activation and inhibitory effects of plasma on LAL test, 0.1 mL serum specimen was diluted (1:10) in apyrogenic sterile water 0.2 mL and Tris-HCl buffer 0.2 mL, boiled for 10 min, and then supernatant obtained by centrifugation at 4 ℃, 5 000 r/min for 10 min was used for detection. The supernatants were coincubated for 25 min at 37 ℃ with LAL, and 3 min after chromogenic substrate was added, reaction was stopped with an aqueous solution of 0.5 g/L naphthyl ethylenediamine. Absorbance was read in a spectrophotometer at 545 nm. The absorbance of a control was subtracted from these absorbances in order to adjust the samples' intrinsic color development. The endotoxin concentration was corrected for dilution and calculated from a standard curve derived from assay of standard (Escherichia coli 0111:B4, 1EU [endotoxin units]=100 pg) supplied by the company. This method was sensitive to 0.03 EU/mL of endotoxin. Depyrogen of detection material was approved by ^{60}Co exposure.

Measurement of intestinal mucus sIgA

Duodenum, jejunum and ileum were excised, cut into 3 segments, opened longitudinally. Intestinal contents were wiped off with bamboo sticks. Intestinal mucus was collected by scraping with glass slides, and dissolved in 1 mL 0.01 mol/L PBS [including 1 mmol/L dithiothreitol (DTT), 100 μg/mL phenylmethyl sulfonylfluoride (PMSF), 10 μg/mL Leupeptin, 10 μg/mL soybean trypsin inhibitor, and 2 μg/mL aprotinin]. The solution was centrifuged at 30 000 *g* at 4 ℃ for 10 min. The supernatant was aspirated and frozen until use.

The supernatants were diluted to 1:400 with 0.01 mol/L PBS for measurement of sIgA concentrations by radioimmunoassay (RIA), based on the protocol supplied with the kit (Biotinge-Tech. Co., Beijing, China). In short, samples were added into glass tubes, incubated with polystyrene-balls which were coated with mouse anti-rat sIgA mAb (Sigma chemical co., St Louis, MO), at 37 ℃ for 2 h, washed 4 times with deionized water, then ^{125}I conjugated goat anti-rat IgA antibody was added. They were kept at room temperature over night. After washed four times, the balls were transferred into another tube for detection. Additionally, a rat myeloma IgA (Zymed, San Francisco, CA) diluted into 15.6 ng/mL, 31.3 ng/mL, 62.5 ng/mL, 125 ng/mL, 250 ng/mL, 500 ng/mL, 1 000 ng/mL, and 2 000 ng/mL, was serially measured for a standard curve. Radionuclide counts were determined in term of disintegrations per minute (dpm) using a gama counter and calibrated by subtracting the background count. Interassay and intraassay coefficients of variation were < 10%. The assay had a sensitivity of 20 pg/mL. Total protein in supernatants was estimated using the Lowry method, simultaneously. Therefore, the concentrations of sIgA from gut mucus were expressed as μg per mg of protein (sIgA μg/mg protein).

Microscopic evaluation

Ileum specimens were dehydrated in progressive concentrations of ethanol, cleared in xylene, and embedded in paraffin. Deparaffinized 4-μm thick sections were stained with hematoxylin-eosin. Glass slides were coded to allow two experienced histopathologists to examine the tissue sections blindly. The degree of intestinal tissue injuries was evaluated using a grading scale from 0 to 8[7]. Grade 0 was defined as

normal mucosa. Pathognomonic for grades 1 to 3 was an increasing subepithelial space of the villi. In grade 4, the villi were denuded, and grade 5 was characterized by loss of the villi. In grade 6, the intestinal crypt layer was also injured, and in grade 7, the entire intestinal mucosa was necrotic. Grade 8 represented transmural infarction.

ELISA for IL-6

Caval blood was collected, centrifuged at 260 r/min for 10 min. Samples were serially diluted with 0.01 mol/L PBS, and IL-6 was determined by sandwich ELISA. The following procedure was based on the protocol supplied with the kit (Bioting-Tech. Co., Beijing, China). Briefly, 96-well plates (Corning Costar, Cambridge, MA) were coated with 100 μL anti-IL-6 mAb diluted in 0.1 mL bicarbonate buffer (pH 8.2) and incubated at 4 ℃ for 48 h. The wells were blocked with PBS containing 10 g/L BSA at room temperature for 1 h. Serial 2-fold dilutions of plasma were added to duplicate wells over night at 4 ℃. Then the wells were incubated with biotinylated anti-IL-6 Ab, at 37 ℃ for 1 h, and then with peroxidase-labeled anti-biotin Ab for 1 h, and developed with ABTS reagent (Sigma). Similarly, a standard curve ranging from 0 to 2 000 pg/mL was plotted using recombinant human IL-6. Absorbance was then read at 410 nm, and the amount of IL-6 in each sample was computed from the standard curve. Interassay and intraassay coefficients of variation were <10%. The assay had a sensitivity of 100 pg/mL.

Statistical analysis

The software package Stata for Windows (Version 6.0) was used for analysis. Results were expressed as mean±SD, except for data of the grading of mucosal injury, which were expressed as median and range. Continuous variables were compared by Student' s *t* test or Wilcoxon- Mann-Whitney rank sum test, whereas the Chi-square test (χ^2 test) was used for comparing proportions. Correlation analysis, univariate and stepwise multivariate logistic regression analysis, with bacterial translocation as the dependent variable, were used to identify factors associated with bacterial translocation to develop a model to predict the probability of incidence. A *P* value of 0.05 or less was considered to statistically significance.

RESULTS

Incidence of bacterial translocation

The overall mortality was 14.7%(22 in 150) during the experiment. The incidence of bacterial translocation was 53.9%(69 in 128) after burn. There were 17 positively cultured strains from abdominal cavity swabs, which were coincident with those strains cultured in organs. The rate of bacterial translocation was 84%, 59%, and 28% on post-burn d 1, 3, and 5, respectively (84% *vs* 59%, *P*=0.02; 84% *vs* 28%, *P*=0.000; 59% *vs* 28%, *P*=0.04), while it was 10% in shame-burn animals.

Univariate analysis

Univariate analysis showed that the levels of plasma endotoxin and interleukin-6, the counts of mucosal fungi and *E. coli*, and the scores of ileum lesion were markedly increased in animals with bacterial translocation compared with those without (*P*=0.000-0.005), while mucus sIgA and the counts of mucosal bifidobacteria were significantly reduced in animals with bacterial translocation compared with those without (*P*=0.000). Moreover, the ratio of bifidobacteria to *E. coli* was decreased significantly from 2 000:1 in animals without bacterial translocation to 10:1 in animals with translocation (Table 1).

Table 1 Univariate analysis of suspected factors for development of bacterial translocation

	BT[1] (*n*=69)	Non-BT[2] (*n*=59)	*t*- or *z*-value	*P*-value
Endotoxin (EU / mL)	0.158±0.0447	0.110±0.0348	6.443	0.000
Microbe flora[3]				
Fungi	3.80±0.8	3.2±0.7	2.859	0.005
E. coli	4.90±1.0	4.0±1.0	5.076	0.000
Bifidobacteria	6.10±0.6	7.3±0.5	6.967	0.000
SigA (μg / mg protein)	55.78±9.81	87.51±10.69	16.857	0.000
Ileo-lesion score	4(2-6)	2(0-4)	9.178	0.000
IL-6 (pg / mL)	871±588	499±308	4.125	0.0001

[1]Animals with bacterial translocation;[2] Animals without bacterial translocation; [3]Mucosal micro-flora ($\log_{10}$ CFU / g tissue).

Table 2 Correlation analysis of data associated with bacterial translocation

	X1	X2	X3	X4	X5	X6	X7
X1	1.0000						
X2	0.7166	1.0000					
X3	0.4586	0.3847	1.0000				
X4	-0.5135	-0.4029	-0.5516	1.0000			
X5	-0.5434	-0.4416	-0.4795	0.7363	1.0000		
X6	0.4834	0.3369	0.3663	-0.6676	-0.7312	1.0000	
X7	0.4807	0.3679	0.2550	-0.3772	-0.3772	0.3549	1.0000

X1: endotoxin levels (EU / mL), X2: counts of fungi ($\log_{10}$ CFU / g tissue), X3: counts of *E. coli* ($\log_{10}$ CFU / g tissue), X4: counts of bifidobacteria ($\log_{10}$ CFU / g tissue), X5: mucous sIgA levels (μg / mg protein), X6: ileal lesion score, X7: IL-6 levels (pg / mL).

Table 3 Independent predictors of bacterial translocation evaluated by multivariate analysis

	Coefficient	Odds ratio (95% CI[1])	*z*-value	*P*-value
X6[2]	3.8182±1.1022	45.52(5.25-394.80)	3.464	0.001
X4[3]	-3.2424±1.2757	0.039(0.003-0.48)	-2.542	0.011
Constant	9.9220±8.7435		1.135	0.256

[1]Confidence interval; [2]Ileal lesion score; [3]Counts of bifidobacteria ($\log_{10}$ CFU / g tissue).

Correlations among experimental findings

As shown at Table 2, there was a significant positive correlation between levels of endotoxin and counts of mucosal fungi and *E. coli*, scores of ileum lesion, and levels of interleukin-6 (r=0.72, 0.46, 0.48, 0.48, respectively. P<0.001), and between mucus sIgA and counts of mucosal bifidobacteria (r=0.74, P<0.001). Moreover, there were strong negative correlations between scores of ileum lesion and counts of bifidobacteria and concentrations of sIgA (r=-0.67, -0.73, respectively. P<0.001), as well as significant negative correlations between counts of mucosal bifidobacteria and levels of endotoxin, and counts of fungi and *E. coli* (r=-0.51, -0.40, -0.55, respectively, P<0.01). Figures 1 and 2 respectively showed the correlation between levels of mucus sIgA and counts of mucosal bifidobacteria, and between counts of mucosal bifidobaacteria and scores of ileum lesion.

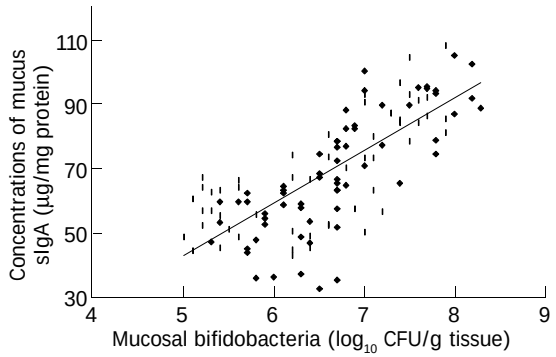

Figure 1 Correlation between counts of mucosal bifidobacteria and intestinal mucous sIgA (r=0.74, P=0.000).

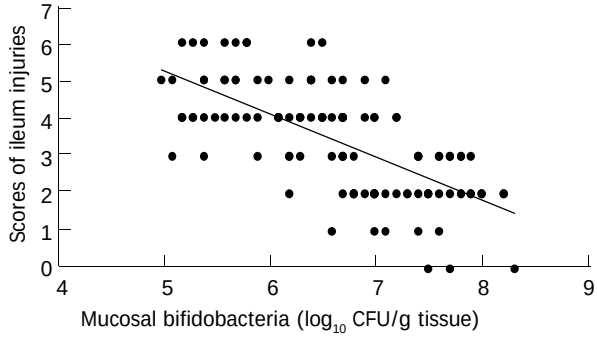

Figure 2 Correlation between counts of mucosal bifidobacteria and scores of ileum injury (r=-0.67, P=0.000).

Multivariate analysis

In multivariate analysis, as shown in Table 3, only two variables in seven parameters remained independent prognosis factors of the development of bacterial translocation associated with postburn injuries. Ileal lesion score was considered as the auxo-action factor, and the counts of mucosal bifidobacteria as the prevention factor. This model was described by the following formula: P (bacterial translocation)=$e^{logit}/(1+e^{logit})$ {logit=9.9220-3.2424 [$\log_{10}$(count of bifidobacteria/g tissue)] +3.8182 (ileal-lesion score)}, with likelihood ratio χ^2=141.28, and the accuracy of the formula for burn rats was (50+67)/128=91% when ileal-lesion score was no less then 4, and the counts of bifidobacteria was no more then 10^6 CFU/g tissue.

DISCUSSION

Intestinal mucosa is a key barrier to prevent the invasion and spread of microorganisms that normally reside within the gut lumen. Under certain conditions, however, bacteria and their products such as endotoxin, can cross this barrier and get access to visceral organs via lymph or blood stream, a process that has been referred to as bacterial translocation. Factors that could promote bacterial translocation included overgrowth of Gram-negative enteric bacilli, impaired host immune defenses, and injury to the intestinal mucosa resulting in increased intestinal permeability[1]. These mechanisms could act in concert to promote synergistically the systemic spread of indigenous translocating bacteria to cause lethal sepsis. Many pathological conditions could evoke bacterial translocation, such as ionization radiation, endotoxemia, dystrophia, peritonitis, renal failure, intestinal obstruction, lesion of mucosa, hemorrhagic shock, long-term fasting, deficiency in secretory IgA, total parenteral nutrition, severe trauma, massive operation, and extensive burn. Recently, some measures were taken to prevent and treat bacterial translocation secondary to trauma with bactericidal/permeability increasing protein, glucagons-like peptide, growth hormone, insulin-like growth factor I, inhibitor of angiotensin II, C1 inhibitor, bombesin, inhibitors of NO synthase, glutamine, lactulose, interleukin-1alpha, probiotics and selective digestive tract decontamination[6,8-17], and their effects on the incidence of bacterial translocation were found to be resulted from ameliorating indigenous flora, strengthening the mucosal barrier, and promoting host systemic or intestinal immunity. However, what role does each of the above mentioned factors play in inciting bacterial translocation is yet to be clarified.

At the early stage of severe burns, there was a considerable change in gut mucosal flora, while only mild alternations occurred in intestinal luminal flora. It is noteworthy that bacterial translocation correlated with the state of colonization in ileum rather than in colon[16]. With these facts in mind, in the present study, we paid more attention to the changes in mucosal microorganisms of the terminal ileal loop. Bifidobacterium, which was the most common species in feces of human[18,19], *E. coli*, which was known to be the most commonly translocated microorganism[4,20,21], as well as fungi, which commonly constituted the pathogens of nosocomial infection in an intensive care unit (ICU), were chosen as representatives of intestinal microflora. Concomitantly, the main pathological changes were located in mucosa of the terminal ileum[22], the scores of lesion in ileum, reflecting the status of mucosa barrier[7], and the levels of mucus sIgA, showing the function of intestinal immunity[23], were determined. The levels of plasma endotoxin and IL-6 were used to evaluate the systemic inflammatory reactions[24,25]. Furthermore, univariate analysis was used to identify the relationship between different variables with occurrence of bacterial translocation, correlation analysis was used for the compliance with the examined variables, and then logistic regression analysis was used to determine the independent predicting factors associated with bacterial translocation.

Bacterial translocation might result from a breach of the intestinal mucosa, as a result of ischemia, atrophy, mechanical injury, *etc*. Repair of injured mucosa depended on the improvement of local microcirculation[26]. During burn shock, with re-distribution of blood circulation, the gastrointestinal suffered a prolonged ischemia, even when systemic hemodynamic parameters were normalized. This condition is known as compensatory covert shock. Our data showed that this hypoxic condition produced injuries to the mucosa of terminal ileum, followed by a significantly increased incidence of bacterial translocation at the early postburn stage. Moreover, the scores of ileal lesion had a significantly positive correlation with serum endotoxin levels. It has been documented that translocated endotoxin could trigger systemic inflammatory response through LBP/CD14 sensibility-increasing system[27], and resulted in an over-release of inflammatory factors such as TNF-α, IL-6, which might further damage intestinal

mucosae[25,27].

There was a marked negative correlation between scores of ileal lesion and mucosal bifidobacteria counts or mucus sIgA. The injury to intestinal mucosa predisposed bacteria to escape from the intestinal wall, while mucosal bifidobacteria and mucus sIgA might be protective against it[15,23,28].

Katouli and colleagues demonstrated that the composition and diversity of gut flora were associated with bacterial translocation, and the proportion and quantity of *E. coli* might influence the incidence of bacterial translocation secondary to hemorrhagic shock[20]. Our results showed that the counts of bifidobacteria decreased by 16-fold, and the counts of *E. coli* and fungi increased respectively by 8- and 4-fold in animals in which bacterial translocation was found. Moreover, the ratio of bifidobacteria to *E. coli* was decreased from 2 000:1 in animals without translocation to 16:1 in animals with translocation. This result might imply that the number of bifidobacteria, especially the ratio of bifidobacteria to *E. coli*, might be of more significance as a marker of bacterial translocation than the quantity and proportion of *E. coli*. Multivariate analysis showed that only the count of mucosal bifidobacteria was the independent predictor for the incidence of bacterial translocation, indicating that bifidobacteria, the predominant anaerobes in the gut, might play a key role in maintaining the biological barrier. The work of others showed that bifidobacteria as a mucosal flora could prevent other intestinal bacteria from adhering to intestinal epithelia through a competitive mechanism, and by producing lactic acid and acetate to create a harmful environment of lowered pH for the growth of *E. coli*, Salmonella, and methicillin-resistant Staphylococcus aureus[28-35]. Our data supported these assertions that administration of bifidobacteria could restore the disrupted gut micro-ecology, which was favorable to the host. As a result, endotoxin release was lessened, and the incidence of bacterial translocation was lowered.

Local immunological defense of the gut was mainly provided by the gut-associated lymphoid tissue (GALT), principally in the form of secretory IgA. sIgA, the predominant immunoglobulin present in mucosal secretions, was the first line of defense on the intestinal mucosal surface[23]. The importance of the mucosal immune system has been extensively recognized, for it relates to the potential pathogenic role of gut flora. Failure of the barrier function of the gut challenged by stress of severe trauma could lead to the translocation of viable enteric bacteria and endotoxin[1]. Our results showed that mucous sIgA was reduced by 56% in animals with bacterial translocation as compared to animals without translocation. However, the concentration of intestinal mucous sIgA was excluded as an independent predictor when examined by multivariate statistical analysis. This might be partially due to the significant positive correlation between the level of sIgA and the count of bifidobacteria. Moreover, the synthesis and excretion of sIgA depended mainly on healthy and stable commensal bacteria[23,30,34]. Overgrowth of Gram-negative bacteria after hemorrhagic shock might also suppress intestinal immunological function and sIgA secretion, augmenting bacterial translocation[36,37]. On the contrary, selective decontamination of the digestive tract (SDD) or supplement of exogenous bifidobacteria could attenuate the incidence of bacterial translocation accompanied by improved intestinal and systemic immunity[15,17,23].

In summary, the development of bacterial translocation was closely associated with overgrowth of pathogens such as fungi and *E. coli*, lowering of intestinal immunologic barrier function, breaching of gut mucosal barrier, and systemic inflammatory reaction subsequent to severe burns. The independent factors related to bacterial translocation were ileal injury score and the counts of gut bifidobacteria. Among them, the score of ileal injury was considered as the augmentative factor, and the counts of mucosal bifidobacteria as the protective factor. Thus specific interventions targeting the high-risk factors, including repair of damaged intestinal mucosa by improving gut microcirculation and improvement of gastrointestinal micro-ecology by increasing the quantity and proportion of bifidobacteria, might be beneficial to the attenuation of bacterial translocation. Moreover, determination of the quantity of bifidobacteria and the ratio of bifidobacteria to *E. coli* in feces might be used to predict the occurrence of bacterial translocation.

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