Tracing method study of bacterial translocation \textit{in vivo}

Fu WL, Xiao GX, Yue XL, Hua C and Lei MP

Subject headings PUC19 plasmid trace; bacterial translocation; restriction map analysis; fluorescence labeling

INTRODUCTION
Endogenesis infection plays an important role in nosocomial infection\cite{1-3}. By studying progress of bacteria translocation from intestinal tract, the concept of gut origin infection has been accepted gradually\cite{4-6}. Because of no ideal tracing method, there were some controversies. In order to solve the problem, the PUC19 plasmid vector tracing method with restriction map analysis and fluorescence labeling method were used to study gut-origin bacterial translocation. According to the characteristic of PUC19 plasmid, a special animal model was designed and two methods were compared.

MATERIALS AND METHODS

Material
PUC19 plasmid vectors (Promega): amplification of plasmid was in LB culture medium containing 100mg/L ampicillin. Fluorescence-labelling bacteria: 1/10000 acridine orange was added in culture fluid of E. coli CMCC 44102. The cells were grown in broth overnight at 37°C. Restriction DNA endonucleases: Hind-III, EcoR-I (Promega Co.). Plasmid was isolated asdescribed by Kado \textit{et al}\cite{3}. A total of 110 male Wistar rats, weighing 226 g±64 g were used. The animals were divided into PUC19 plasmid group and fluorescence-labeling one. The animals in fluorescence labeling group were sacrificed and examined at 4, 12, 24 and 48 h postburn with 10 rats at each time point, the normal control group contained 10 rats, with a total of 50 rats. The rats in PUC19 group were treated the same as those described above, another 10 rats were added at the time point of the 12th day postburn.

Methods
Fluorescence labeling group: 10^{12}/L fluorescence-labelling E.coli were introduced into the stomach of rats by gastric tube. After 8 hours the animals had 30% TBSA (total burned surface area) full thickness burns. Three mL/100 g body weight saline was injected into rat’s abdominal cavity for anti-shock. The rats were sacrificed at 6, 12, 24 and 48 h after burn. Mesentery lymph nodes (MLN), liver and subeschar tissue were collected by aseptic technique. The homogenates were divided into two parts, the first part cultured for enumerating microorganisms and second part examined under fluorescence microscope.

PUC19 plasmid tracer group: before burn, animals drank 300 mg/L ampicillin fluid for 3 days to “clean up” the intestinal tract. The PUC19 plasmid vector was introduced into the stomach by way of gastric tubing. Then the animals were again given ampicillin fluid (100 mg/L ampicillin) and examined to confirm whether PUC19 plasmid vector had colonized in the rat’s intestinal tract. The animals were inflicted with 30% TBSA third degree burn and sacrificed at 6, 12, 24, 48 h and 12 d postburn. The methods were the same as those described above. Homogenates of mesenteric lymph nodes, liver, and subeschar tissues were incubated in LB broth containing 100 mg/L ampicillin (18 hours) at 37°C with shaking at 50 min^{-1}-100 r.p.m.

Plasmid isolation of the bacteria was made according to Kado’s method. The product was digested 1h at 37°C by restriction enzymes EcoRI and Hind III. Ten g/L agarose gel with well sufficient to accommodate 40 µL samples was prepared and the gel was stained with ethidium bromide. Photographs of gel were taken and positioned over an ultra-violet (UV) ray source.

RESULTS

Fluorescence-labeling bacteria
Labeling bacteria were found to pass through damaged intestinal mucosal barrier and escaped from cleansing effect of liver and lymph nodes and finally reached the subeschar area.
Detection rates of fluorescence-labeling bacteria from mesenteric lymph nodes, liver and subeschar tissues were enumerated (Table 1). The quantity and rate of bacteria by fluorescence-labeling at different time points after burn are shown in Table 2.

| Organ and tissue          | No. of samples | Positive rate (%) | No. of FB/*10×100 |
|---------------------------|----------------|-------------------|-------------------|
| Mesenteric lymph nodes    | 40             | 38(95.0)          | 3.8               |
| Liver                     | 40             | 23(57.5)          | 2.1               |
| Subeschar                 | 40             | 13(32.5)          | 1.1               |

Table 2 Quantity and rates at different periods after burn

| Group         | Mesenteric lymph node | Liver |
|---------------|-----------------------|-------|
| t/h           | Rate Quantity(cfu/g)  | Rate Quantity(cfu/g) |
| Burn 6        | 100 3.0×10⁴           | 70 2.1×10⁴ |
| 12            | 100 5.5×10⁴           | 70 3.1×10⁴ |
| 24            | 90 7.4×10⁴           | 50 2.6×10⁴ |
| 48            | 70 8.2×10³           | 40 1.5×10³ |
| Normal        | 60 8.0×10⁵           | 0 0     |

**PUC19 plasmid tracing**

Bacteria that resist to ampicillin (AMP) could be separated from mesenteric lymph nodes, liver and subeschar tissues after burn. Isolation of plasmid and restriction enzymes analysis indicated that bacteria resistant to ampicillin that separated from intestinal and subeschar tissue had the same DNA restriction map (Figure 1).

The detected quantity and positive rate of PUC19 tracing bacteria after burn are shown in Table 3. The positive rate of PUC19 bacteria in early and late stages after burn is shown in Table 4.

**DISCUSSION**

In recent years, more and more studies in gut-origin infection have been reported. Gut-origin infection as an important way of infection in burn had been established. But there were still some disputes about its verification by tracing methods [7-15]. In the past, these included the fluorescence and isotopes labeling methods. PUC19 plasmid was constructed in the 1980s, and was used extensively in molecular cloning and discrimination of gene recombination [16]. The characteristics of the PUC19 plasmid were: (1) plasmid vector carried ampicillin resistance gene; (2) the plasmid possessed polyclonal restriction endonuclease sites, panning positive bacteria became easier to be discriminated; and (3) it lacked nic/bom site, hence gene transduction from conjugate of bacteria was impossible. These features indicated that PUC19 plasmid could be an ideal tracer for studying endogenous bacterial translocation.

Comparing the results of the two tracing methods used in our experiment, we could find the positive rate of fluorescence-labeling organisms was higher than that by PUC19 plasmid tracing method in bacteria reaching mesenteric lymph nodes and liver after serious burn. Because many factors can cause nonspecific reaction in fluorescence-labeling method, some false positive results may be present, but we think that fluorescence-labeling bacteria tracing method is still useful for gut bacterial translocation study, although not for quantitation study and long period study.

**REFERENCES**

1. Jones WG, Barber AE, Minei JP, Fahey TJ, Shires T. Antibiotic prophylaxis diminishes bacterial translocation but not mortality in experimental burn wound sepsis. *J Trauma*, 1990;30:737-739
2. Wu YZ, Wu JS, Lai DN, Ma QJ, He ZS, Gao DM. Morphology of gastric mucosal bleeding site in rats with chronic portal
hypertension. *Shijie Huaren Xiaohua Zazhi*, 1998;6:744-746
3 Fu WL, Xiao GX, Yu PW, Zhou LX, Yan JC, Qin XJ. Changes of circulating LPS and cytokines in burned patients after anti endotoxin therapy. *Zhonghua Yiue Zazhi*, 1996;76:355-358
4 Fleming RYD, Zeigler ST, Walton MA, Herndon DN, Heggers JP. Influence of burn size on the incidence of contamination of burn wounds by fecal organisms. *J Burn Care Rehabil*, 1991;12:510-514
5 Wu YZ, He ZS, Wu JS, Lai DN, Ma QJ, Gao DM. Starvation on gastric mucosal hemorrhage in rats with liver cirrhosis and portal hypertension. *Shijie Huaren Xiaohua Zazhi*, 1998;6:747-748
6 Fu WL, Xiao GX, Zhou LX, Yu PW, Yan JC, Qin XJ. Cloning expression and identification of single chain antibody against lipid A of bacterial endotoxin. *Zhonghua Zhengxing He Shaoshang Waike Zazhi*, 1997;13:94-96
7 Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol*, 1981;145:1365-1373
8 Wu YZ, Wu JS, Lai DN, Ma QJ, He ZS, Gao DM. Relationship between ectasias in gastric microvascular system and cytokinetics in gastric mucosal epithelial cell group in PHG-MH rats. *Shijie Huaren Xiaohua Zazhi*, 1998;6:752-754
9 Fu WL, Xiao GX, Xiao H, Zhou LX. Therapeutic effects of anti-endotoxin antibodies and antibiotics on endotoxemia in patients with severe burns. *J MedColl PLA*, 1997;12:223-226
10 Mansion WL, Coenen JM, Klasen HJ. Bacterial translocation in experimentally burned mice with wounds colonized by *Pseudomonas aeruginosa*. *J Trauma*, 1992;33:654-657
11 Chu YK, Wu JS, Ma QJ, Gao DM, Wang X. Plasma TNF levels during the formation of liver cirrhosis and portal hypertension in rats. *Shijie Huaren Xiaohua Zazhi*, 1998;6:755-756
12 Kohn FR, Ammons WS, Horwitz A, Grinnah L, Theofan G, Weickmann J, Kung AHC. Protective effect of a recombinant aminoterminal fragment of bactericidal/permeability increasing protein in experimental endotoxemia. *J Infect Dis*, 1993;168:1307-1309
13 Jones WG, Minei JP, Barber AE, Rayburn JL, Fahey TJ, Shires III GT, Shires GT. Bacterial translocation and intestinal atrophy after thermal injury and burn wound sepsis. *Ann Surg*, 1990;211:399-405
14 Manson WL, Coenen JMFH, Klasen HJ, Horwitz EH. Intestinal bacterial translocation in experimentally burned mice with wounds colonized by *Pseudomonas aeruginosa*. *J Trauma*, 1992;33:654-658
15 Pfirrmann RW, Leslie GB. The anti endotoxin activity of taurocholic acid in experimental animals. *J Appl Bacteriol*, 1979;46:97-102
16 Vieira J, Messing J. The pUC plasmids, an M13 mp7 derived system for in sertion mutagenesis and sequencing with synthetic universal primers. *Gene*, 1982;19:259-268

Edited by Wu XN and Ma JY
Proofread by Miao QH