# Bacterial Translocation from the Gastrointestinal Tracts of Multiple Low-Dose Streptozotocin-Treated Mice and Non-Obese Diabetic Mice

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Bacterial translocation is defined as the passage of viable indigenous bacteria from the gastrointestinal(GI) tract through the mucosal epithelium to the mesenteric lymph nodes (MLN) and other sites. It is the important early event in the pathogenesis of certain opportunistic infections [2]. Bacterial translocation of indigenous bacteria has been detected in diabetic mice induced by single high-dose of streptozotocin(STZ) [2, 5]. Diabetes may allow certain indigenous bacteria to pass from the GI tract to other organs and to cause opportunistic infections. However, diabetes induced in mice by single high-dose of STZ has been considered as a "toxic" diabetes [3]. Thus, it is not clear whether hyperglycemia induced by STZ causes bacterial translocation from the GI tract or some toxic effects of STZ cause it. On the other hand, multiple low-dose injections of STZ in mice produce pancreatic insulitis and diabetes mellitus, and then these mice are thought to be not a "toxic" diabetes but a model of human type I (insulin-dependent) diabetes same as non-obese diabetic (NOD) mice [6, 7]. Our purpose in this study was to observe the translocation of indigenous bacteria from the GI tract in some strains of mice treated with multiple low-dose STZ and NOD mice which had develop spontaneous diabetes without any artificial treatment such as STZ.

Female BALB/c, CBA/J, ICR and NOD mice maintained in Laboratory Animals Breeding Facilities, Osaka University Medical School were used for the present study. The mice were housed under conventional conditions with controlled temperature (23°C) and lighting (12 hr light-dark cycles). The mice were fed mouse pellet (NMF: Oriental Yeast Co., Tokyo, Japan) and given hyperchlorinated water (10 ppm sodium hypochlorite) *ad libitum*. Urinary glucose in the NOD mice was tested with Tes-Tape (Eli Lilly & Company, Indianapolis, Ind., U.S.A.) once a week. BALB/c, CBA/J, and ICR mice, aged 7 to 10 weeks, received daily five intraperitoneal injections of STZ (Sigma Chemical, Co., St. Louis, Mo., U.S.A. 40 mg/kg per body weight) dissolved in a citrate buffer solution, pH 4.2 [6]. These mice were tested for bacterial translocation 2 weeks after the completion of STZ injections. The diabetic NOD mice, aged 140 to 239 days were used for the same experiment 2 weeks after the detection of glycosuria. Mice were anesthetized with ether and urinary glucose was tested with Tes-tape. Blood was obtained by heart punctures and blood glucose was determined with TOECHO II (Kodama, Co., Ltd., Tokyo, Japan). The blood (0.5 m*l*) was transferred into 20 m*l* of tryptic soy broth (TSB: Eiken, Co., Ltd., Tokyo, Japan) and incubated for 48 hr at 37°C, and then subcultured on MacConkey agar (Eiken). An incision was then made with sterile instruments through the skin and peritoneum of the abdomen. The exposed viscera were swabbed with sterile, cotton-tipped applicator sticks. The sticks were cultured in 10 m*l* of TSB to detect any bacteria translocating to the peritoneal cavity. The MLN and spleen were removed aseptically, and each placed in grinding tubes containing 1.0 m*l* of TSB. The liver was then removed and placed in grinding tube containing 3.0 m*l* of TSB. The cecum was removed and placed in grinding tube containing 9.0 m*l* of TSB. The organs were weighed and then homogenized with Teflon grinders. Portions (0.2 m*l*) of the MLN, liver and spleen homogenates were plated onto each of two MacConkey agar plates and incubated for 20 hr at 37°C. One milliliter of TSB was added to the remaining these homogenates. They were incubated overnight at 37°C and then subcultured on MacConkey agar. The cecal homogenate was diluted serially in phosphate buffered saline (PBS) and cultured on MacConkey agar plates for the counts of viable bacterial numbers. The bacteria were identified with the API-20E system (Analytab Products, Plainview, NY., U.S.A.).

STZ-injected mice showed glycosuria and hyperglycemia (Table 1). Blood glucose values for STZ-treated mice were significantly elevated compared with non-treated control mice. Blood glucose values of STZ-treated ICR mice tended to be higher than those of STZ-treated BALB/c and CBA/J mice, though there was no significant difference among those of ICR, BALB/c and CBA/J mice. In NOD mice, blood glucose values of diabetic mice were significantly elevated in comparison with those of non-diabetic mice. The cecal population levels of indigenous enteric bacteria in STZ-treated BALB/c and ICR mice were significantly greater than those of control mice. The values of STZ-treated CBA/J mice were slightly higher than those of control mice. Whereas, there is no difference between diabetic and non-diabetic NOD mice in the number of enteric bacteria in cecum.

In the STZ-induced diabetic mice, the viable indigenous bacteria were translocated to 18% of livers of total diabetic mice, which was 20% of BALB/c mice, 14% of CBA/J mice, and 20% of ICR mice (Table 2). Cultures of

Table 1. Blood glucose levels and cecal population levels of enteric bacteria in diabetic mice

| Mice | No. Animals | Blood glucose (mg/dl) | Enteric bacteria in cecum($log_{10}$/g) |
|---|---|---|---|
| BALB/c | | | |
| control | 5 | 130±10 | 4.88±0.79 |
| STZ-treated[a)] | 5 | 377±180[b)] | 6.54±1.74[c)] |
| CBA/J | | | |
| control | 5 | 181±12 | 4.80±0.47 |
| STZ-treated | 7 | 372±67[b)] | 5.42±0.73 |
| ICR | | | |
| contorl | 6 | 118±24 | 4.85±0.60 |
| STZ-treated | 10 | 426±110[b)] | 6.22±1.92[c)] |
| NOD | | | |
| non-diabetes | 10 | 205±76 | 5.12±1.01 |
| diabetes | 9 | 511±179[b)] | 5.10±0.70 |

Data are expressed as mean ± standard deviation.
a) Streptozotocin treated.
b) Significantly ($p<0.01$) different from the mean value of controls.
c) Significantly ($p<0.05$) different from the mean value of controls.

Table 2. Bacterial translocation of indigenous bacteria from GI tract in diabetic mice

| | Translocation incidence[a)] | | | | |
|---|---|---|---|---|---|
| Mice | MLN | Liver | Spleen | Blood | Peritoneum |
| BALB/c | | | | | |
| control | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| STZ-treated[b)] | 0/5 | 1/5 | 0/5 | 0/5 | 0/5 |
| CBA/J | | | | | |
| control | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| STZ-treated | 0/7 | 1/7 | 0/7 | 0/7 | 0/7 |
| ICR | | | | | |
| control | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| STZ-treated | 1/10 | 2/10 | 0/10 | 0/10 | 0/10 |
| NOD | | | | | |
| non-diabetes | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| diabetes | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 |

a) Incidence is expressed as the number of organs giving positive cultures / the number of organs tested.
b) Streptozotocin treated.

the blood, spleen and peritoneum were negative. Only 1 of 22 of MLN of STZ-treated mice tested had viable bacteria. These indigenous bacteria translocating to the liver and MLN were identified *Escherichia coli*. These bacteria were considered to be indigenous bacteria from the GI tract, because no viable bacteria were detected in any organs when STZ-treated mice were given drinking water supplemented with antibiotics (streptomycin and bacitracin). No viable bacteria were isolated from MLN, liver, spleen, blood and peritoneum of control mice. On the other hand, no viable bacteria were isolated from tested organs both of non-diabetic and diabetic NOD mice.

Multiple low-dose STZ-treated mice which induce pancreatic insulitis are thought to be an animal model for human Type I (insulin-dependent) diabetes [6]. These permanent hyperglycemia and insulitis have been argued about the immune pathogenesis, whereas diabetes induced by single high-dose STZ treatment has been considered as a "toxic" diabetes [3]. Diabetes induced in mice by single high-dose of STZ exhibited the increase in the number of aerobic gram-negative bacteria in cecal floras and the translocation of indigenous bacteria from their GI tracts to their MLN or several other organs [2, 4, 5]. In our study, we also detected bacterial overgrowth in the ceca and translocation of *E. coli* from the GI tract to the livers and MLN in multiple low-dose STZ-treated mice similar to those found in single high-dose STZ-treated mice. Although the reasons why only the *E. coli* was detected from various organs could not be elucidated, it has been known that *E. coli* is the major pathogen of postoperative bacteremia in diabetic [1]. In this study, we did not culture anaerobic organisms because, in general, aerobic bacteria were the causative organism in infections

of diabetic patients [1, 8]. Further study is necessary to elucidate whether anaerobic bacteria are capable of translocation in multiple low-dose STZ-treated and NOD mice.

There is a strain difference of mice in the susceptibility to the diabetogenic action of STZ [9]. BALB/c and CBA/J mice are the resistant strains for STZ treatment. However, in this study there was no difference among mouse strains treated with STZ as to in view of the bacterial translocation. While, spontaneous diabetic NOD mice which showed severe hyperglycemia exhibit neither bacterial overgrowth in the ceca nor *E. coli* translocation to other tested organs. These results suggest that some other toxic effects of STZ induce *E. coli* translocation from the GI tract independent of its diabetogenic actions.

In conclusion, diabetes in mice induced by multiple low-dose STZ seems to be a "toxic" diabetes as single high-dose STZ-treated mice rather than autoimmune diabetes resembling human insulin-dependent diabetes mellitus in terms of bacterial translocation.

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