Effect of Age on Bacterial Translocation from the Gastrointestinal Tract in Mice

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To clarify the effects of age on bacterial translocation from the gastrointestinal tract, mice at the age of 1, 2, 4, 6, 12, and 15 months were antibiotic-decontaminated for 4 days and then inoculated orally with streptomycin-resistant *Escherichia coli* C25. Mice treated with cyclophosphamide and untreated controls were tested for bacterial translocation to the mesenteric lymph nodes (MLN) 2 days later. The population levels of *E. coli* C25 in cyclophosphamide-treated and untreated mice were approximately $10^{9.3}$ and $10^{9.5}$ per gram of cecum, respectively, at each tested age. There were no significant differences in the incidence of translocation of *E. coli* C25 to MLN at any of the tested ages, whereas the number of *E. coli* C25 detected in MLN was higher in young mice than in aged mice in both the cyclophosphamide-treated and untreated groups. These findings suggest that bacterial translocation from the GI tract may be a more important problem in young animals than in aged animals. — KEY WORDS : aging, bacterial translocation, mice

Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal (GI) tract through the mucosal epithelium to other sites [1, 5, 16]. The translocation of intestinal bacteria appears to be facilitated in young animals during the newborn period [6, 12]. The greater incidence of bacterial translocation in young animals is thought to be due to still incomplete development of the intestinal mucosal barrier [14].

However, the effects of age on bacterial translocation from the GI tract after the completion of the intestinal mucosal barrier and the incidence of bacterial translocation in aged animals are not known. Furthermore, immunosuppressive agents such as cyclophosphamide (CY) promote bacterial translocation [2, 3], the effects of treatment with these agents on

bacterial translocation at different ages is also unknown. To answer these questions, we examined the bacterial translocation from the GI tract in mice at 1, 2, 4, 6, 12, and 15 months of age. In addition, we investigated the bacterial translocation in mice treated with CY at the same ages. These experiments were carried out by colonizing the intestinal tracts of the mice with extremely large numbers of *Escherichia coli* and then determining the amount of translocated *E. coli* in the mesenteric lymph nodes (MLN) [8, 10]. In both the CY-treated and the untreated mice, the number of *E. coli* detected in MLN was higher in young mice than in aged mice, although the incidence of translocation did not differ at any age during the course of the experiment.

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Materials and Methods

Mice : Specific pathogen-free (SPF) male ICR mice were obtained from our breeding colony at 1, 2, 4, 6, 12, and 15 months of age. The colony was produced from several mice originally purchased from Charles River Japan Inc. The mice were housed under barrier-sustained conditions with controlled temperature (22°C), and lighting (12-hr light/dark cycles). They were fed mouse pellets (CRF-1 : Oriental Yeast Co. Ltd., Tokyo) and autoclaved water was given *ad libitum*.

Cyclophosphamide (CY) treatment : Cyclophosphamide (Endoxan® ; 200 mg/kg, Shionogi & Co. Ltd., Osaka) was injected once intraperitoneally at various ages. The mice were tested for bacterial translocation 1 week after the injections.

Antibiotic decontamination : Cyclophosphamide-treated and untreated mice continuously received 4 mg/ml of streptomycin (Meiji Seika Co. Ltd., Tokyo) and 4 mg/ml of bacitracin (Sigma Chemical Co. St. Louis, Mo., U. S. A.) in their drinking water, which was available *ad libitum*, for four days, as described previously [10]. These antibiotic-decontaminated mice were then inoculated orally, using feeding needles, with 3×10^8 streptomycin-resistant *E. coli* C25 [11]. The *E. coli* C25 was obtained from Dr. Berg, Louisiana State University, Shreveport, La., U. S. A. The mice continued to receive streptomycin and bacitracin in their drinking water and they were examined for bacterial translocation 2 days after receiving *E. coli* C25.

Translocation of *E. coli* C25 : After mice were killed by cervical dislocation, sterile instruments were used to make an incision through the skin and peritoneum of the abdomen. The mesenteric lymph nodes (MLN) and ceca were removed and placed in grinding tubes containing 1 ml and 9 ml of tryptic soy broth (TSB : Eiken Co. Ltd., Tokyo), respectively. The organs were weighed and then homogenized with a Teflon grinder. The cecal homogenate was diluted serially with phosphate buffered saline (PBS), pH 7.4. Portions (0.2 ml) of the MLN and diluted cecal samples were spread on MacConkey agar plates (containing 1 mg/ml of streptomycin sulfate) followed by incubation for 20 hr at 37°C. Bacteria were identified

according to the enteric identification system (API-20 E ; Analytab Products, Plainview, NY., U. S. A.).

Results

Cecal weight/body weight ratio : The cecal weight/body weight ratio in both 1-month-old CY-treated and untreated groups was higher than that at other tested ages (Fig. 1). There

Fig. 1. Changes observed with age in cecal weight to body weight ratio in mice untreated (○) or treated (●) with cyclophosphamide (CY). Each value represents the mean $\pm$ standard deviation. Numbers of tested mice are shown in Tables 1 and 2.

Fig. 2. Changes observed with age in the number of *Escherichia coli* C25 colonizing the cecum following inoculation of mice untreated (○) or treated (●) with cyclophosphamide (CY). In CY-treated mice, *Enterobacter cloacae* (▲) was observed in the cecum at 12 and 15 months of age. Each value represents the mean $\pm$ standard deviation. Numbers of tested mice are shown in Tables 1 and 2.

was a significant difference between this ratio at 1 month of age and the ratios at other tested ages; however, after the age of 2 months, there were no significant differences between tested ages in either CY-treated or untreated mice. There were also no significant differences between CY-treated mice and untreated mice at any age.

Number of *E. coli* C 25 in the cecum : *E. coli* C 25 colonized in the ceca of CY-treated and untreated mice at approximately $10^{9.3}$ and $10^{9.5}$ per gram, respectively, at each tested age (Fig. 2). *Enterobacter cloacae* was present in the ceca of 2 of the 4 CY-treated mice at 12 months of age, and in 3 of the 5 CY-treated mice at 15 months of age at levels of $10^{8.4}$ and $10^{8.5}$ per gram, respectively. One of the 5 mice treated with CY at 12 months of age died 1 day after

Fig. 3. Changes observed with age in the incidence of *Escherichia coli* C 25 translocation to the mesenteric lymph nodes (MLN) in mice untreated (▨) or treated (■) with cyclophosphamide (CY). Numbers of tested mice are shown in Tables 1 and 2.

Table 1. Changes observed with age in the number of *Escherichia coli* C25 (*E. coli* C25) detected in mesenteric lymph nodes (MLN) in untreated mice

Age (months)	No. of mice ^{a)}	Number of <i>E. coli</i> C25 ^{b)}
1	8/8	22 (5–53)
2	8/8	14 (3–35)
4	6/7	18 (10–48)
6	3/3	9 (3–13)
12	6/7	8 (3–20)
15	5/5	7 (3–13)

a) Positive organs/tested organs. These results are also shown in Fig. 3.

b) Values are the mean of the number of viable *E. coli* C25 per MLN. The values in parentheses show the range.

Table 2. Changes observed with age in the number of *Escherichia coli* C25 (*E. coli* C25) and *Enterobacter cloacae* detected in mesenteric lymph nodes (MLN) in cyclophosphamide-treated mice

Age (months)	No. of mice ^{a)}	Number of bacteria in MLN	
		<i>E. coli</i> C25 ^{b)}	<i>Enterobacter cloacae</i> ^{c)}
1	6/6	827 (6–4,750)	— ^{d)}
2	4/5	1,157 (60–4,400)	—
4	4/5	2,014 (5–8,025)	—
6	3/3	157 (13–440)	—
12	3/4 ^{e)}	250 (3–718)	19 (15,23) ^{f)}
15	4/5	179 (5–610)	16 (13–20) ^{g)}

a) Positive organs/tested organs. These results are also shown in Fig. 3. b) Values are the mean of the number of viable *E. coli* C25 per MLN. The values in parentheses show the range. c) Values are the mean of the number of viable *Enterobacter cloacae* per MLN. The values in parentheses show the range. d) Not detected. e) One of the original 5 mice died 1 day after *E. coli* C25 inoculation. f) Two of the 4 mice were positive. g) Three of the 5 mice were positive.

E. coli C 25 inoculation.

Incidence of *E. coli* C 25 translocation to mesenteric lymph nodes (MLN): No significant differences were observed in the incidence of translocation at any age tested in either CY-treated or untreated mice (Fig. 3). Furthermore, there were no differences in the incidence of translocation between CY-treated and untreated mice.

Number of *E. coli* C25 detected in MLN: The number of *E. coli* C25 detected in MLN decreased slightly with age, although there were no significant differences between the incidence of *E. coli* C 25 translocation at any age tested (Table 1). The same trend was observed in CY-treated mice (Table 2). In these mice, until 4 months of age, the number of *E. coli* in MLN was about 2 to 3 times higher than that after 6 months of age in untreated mice. Likewise, the number of *E. coli* in the MLN of mice under 4 months of age was about 10 times higher than that after 6 months of age in the CY-treated group. Mice treated with CY had approximately 10- to 100- fold more *E. coli* in their MLN than untreated mice at all ages. *E. cloacae* was detected in MLN when *E. cloacae* was present in the cecum.

Discussion

The indigenous gut flora is thought to be an important source of potential septicemia in immunocompromised patients, such as burn patients [2, 3, 8]. During the aging process, normal immune responses diminish and sensitivity to infection increases. Therefore, senescent mammals may be considered to be in an immunocompromised condition. Hyde *et al.* [7] reported that in the cecal ligation and puncture procedure (CLP) model, aged mammals were more sensitive than younger mammals to the lethal effects of invasive bacterial infection with indigenous gut flora. However, the effects of age on invasive bacterial infection with indigenous gut flora in mice with intact intestinal barriers are not known.

The passage of viable indigenous bacteria from the gastrointestinal (GI) tract through the intact epithelial mucosa to the mesenteric lymph nodes (MLN) and other organs is called bacterial translocation. Bacterial translocation is an important early event in opportunistic infection with indigenous gut flora. Some

researchers [6, 12] have reported that bacterial translocation might be increased in young animals during the neonatal period. The incomplete development of the intestinal mucosal barrier may facilitate bacterial translocation from the GI tract to the MLN [14]. Furthermore, certain neonates absorb by pinocytosis various macromolecules, such as maternal immunoglobulins, through the intestinal epithelium. After gut closure, which occurs at 17-21 days of age in mice, these macromolecules are no longer absorbed [4, 13]. Thus, indigenous bacteria may translocate more readily from the GI tract of neonates prior to gut closure than from the GI tract of adult animals. In this study, we investigated the effects of age on bacterial translocation in mice after gut closure, and our data indicated that the number of *E. coli* detected in MLN was higher in young animals than in aged animals. In CY-treated mice, the same trend was observed, although the number of *E. coli* detected in MLN was 10- to 100- fold higher than the number in untreated mice at each age. This difference might be caused by age-related changes in intestinal function rather than age-related changes in intestinal microbial ecology, since the cecal weight to body weight ratio reached a stable value after 2 months of age, and the number of inoculated *E. coli* in the cecum did not vary at any tested age. Although the postweaned mice already possess histologically complete intestinal mucosa and occur gut closure, the number of translocated bacteria decreased slightly with age. It is proposed that a few epithelium cells possessing pinocytosis to absorb macromolecules remain after gut closure, and then these cells decrease with age.

On the other hand, it has been hypothesized by Wells *et al.* [15] that intestinal bacteria could be ingested by gut macrophages, and then translocated to other sites. If their hypothesis is true, the decreased number of translocated bacteria in aged mice relative to that in younger mice might be explained by an age-related decrease in the number or function of gut macrophages available for bacterial translocation. However, the effect of age on functions of macrophage is unknown.

Enterobacter cloacae was observed in CY-treated mice at 12 and 15 months of age. *E. cloacae* was not observed in microbial monitor

mice treated with antibiotics and reared in the same cage. *E. cloacae* is usually thought to be non-pathogenic, but, mice contaminated with this organism died earlier than expected after irradiation [9]. Further studies are necessary to determine the mechanisms of *E. cloacae* colonization in the GI tract of aged mice treated with CY.

In this study, we did not find any age-related promotion of bacterial translocation from the GI tract. Contrary to our expectation, the number of *E. coli* detected in MLN in young mice was higher than that in aged mice. However, incidence of translocation did not differ at any age tested. These findings suggest that bacterial translocation from the GI tract may be more important in young animals than in aged animals.

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バクテリアトランスロケーションにおける 加齢の影響

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腸管固有細菌が腸間膜リンパ節、その他臓器へ移行する現象であるバクテリアトランスロケーションの加齢による変動を検索した。1 から15ヵ月齢のマウスを抗生物質で腸管内浄化後、大腸菌を投与し、その2日後に盲腸内および腸間膜リンパ節内大腸菌を検索した。さらに免疫抑制処置（サイクロフォスファミド投与）群を置き、同様に検索した。盲腸内大腸菌数は、月齢にかかわらず、免疫抑制処置群および対照群で各々 $10^{9.3}/g$, $10^{9.5}/$

g , とほぼ一定の値を示した。また、投与大腸菌の腸間膜リンパ節への移行の頻度は、各月齢および免疫抑制処置によって差は認められなかった。しかし、腸間膜リンパ節から検出された投与大腸菌数は、免疫抑制処置群、無処置群ともに若齢マウスの方が老齢マウスより高い傾向が認められた。以上の成績からバクテリアトランスロケーションは老齢期よりも若齢期で、より重要であると考えられた。