Development of Intestinal Flora of Human-Flora-Associated (HFA) Mice in the Intestine of their Offspring

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Abstract: Development of intestinal flora in newborn human-flora-associated (HFA) mice was compared with that in newborn conventional (CV) mice. Facultative anaerobes were detected from the first day after birth in both CV and HFA mice but anaerobes were not detected in the first week. Anaerobes rapidly increased from the 2nd week after birth and became predominant in newborn intestine. Most of the intestinal bacteria in adult CV and HFA mice were colonized in the intestine of CV and HFA mice, respectively, within 3 weeks after birth. The human intestinal flora established in the intestine of HFA mice finally reproduced without any remarkable change in composition in the intestine of newborn HFA mice. The development of intestinal flora in HFA mice was similar to that in CV mice but not that in human infants. These results indicated that human flora associated in HFA mice could be transferred from mothers to their offspring although HFA mice could not simulate the development of intestinal flora of the human infant.

Key words: Germfree, human-flora-associated mice, infant, intestinal flora

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Introduction

By inoculating germfree (GF) animals with human fecal bacteria, it is possible to produce human-flora-associated (HFA) animals harboring intestinal flora of human origin in their intestine. Since the composition of intestinal flora of HFA animals is similar to that of human fecal flora [4, 5, 14], these animals have been employed in clinical studies [16] and in studies related to the effects of dietary components on human intestinal flora [6]. Whether the floral composition of HFA mice can be transferred stably from mothers to their offspring is an important subject when HFA mice are used as an experimental animal. Although it has been reported that the development of intestinal flora of newborn mice [8, 15] and human infants [1, 10] is different, that of HFA mice is still unknown.

In the present study, we investigated the development of intestinal flora of newborn HFA mice and compared it with that of conventional (CV) newborn mice and that of human infants. Finally, we attempted to determine if intestinal flora can be transferred to the offspring by breeding of HFA mice.

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Materials and Methods

*Animals and diet:* CV and GF BALB/cA (Jic) mice were bred and maintained in our laboratory. Two groups of HFA mice (HFA mice A and B) were prepared by inoculating with $10^{-3}$ dilutions of feces from 23- and 26-year-old healthy males. The procedure for preparation of HFA mice was as reported previously [5, 6]. All animals were kept in the same way as GF mice in flexible vinyl isolators and fed CMF pelleted diet (Oriental Yeast Co.) sterilized by $\gamma$-irradiation at 50 kGy. They were mated and their offspring were weaned at 3 weeks of age.

*Sampling schedules:* Three to 5 samples were processed for each sampling period. One, 3 and 5 days after birth, infants were sacrificed by $CO_2$ gas, and the whole intestine with contents was removed for microbiota examination. Seven, 10 and 14 days after birth, only the large intestine was removed, and 21, 35 and 60 days after birth, feces were collected from each mouse. Feces from their mothers were collected before mating.

*Bacteriological procedures:* Bacteriological procedures were essentially the same as those described by Mitsuoka et al. [11, 13], and Itoh and Mitsuoka [7]. After serial 10-fold dilution of homogenized intestines or feces under $O_2$-free $CO_2$ gas flow, the diluted samples were spread on the surface of 10 selective and four nonselective media. Media and cultural conditions are summarized in Table 1. Medium 10 was used in the 'plate-in-bottle' method [12]. Mouse cecal extract was added to medium 10 for CV mouse samples. Bacterial numbers were expressed as log$_{10}$ counts of viable bacteria per gram wet weight of intestine or feces. Facultative anaerobes were indicated with open symbols and obligate anaerobes were indicated with closed symbols in Figs. 1 to 3.

Results

*Development of intestinal flora of CV mice:* The development of intestinal flora of CV mice is shown in Fig. 1. Only facultative anaerobes were detected in the first week of life. Lactobacilli became the most predominant bacteria on 5th day and remained at high concentrations throughout the observation period. Staphylococci gradually increased their number to about $10^{9}$ g-feces. Other aerobic bacterial groups increased rapidly in the first 2 weeks of life and then decreased. Aerobic Gram-negative rods could not be detected on the 21st day and thereafter. Obligate anaerobes, including bacteroides, eubacteria, clostridia and fusiform-shaped bacteria, were detected from 10 days after birth, gradually increased their numbers and became the predominant bacteria by the 21st day of life. Stabilized intestinal flora of CV mice was established within 3 to 5 weeks after birth.

<table>
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<td>Medium 10</td>
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<td>'Plate-in-bottle' method</td>
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<td>PD agar</td>
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Development of intestinal flora of HFA mice: The development of intestinal flora of two groups of HFA mice is shown in Figs. 2 and 3. Although bifidobacteria did not colonize in the mothers and newborn of HFA mice B, bacterial groups of intestinal flora in HFA mice B developed in their newborns in a similar manner to those in HFA mice A. Only facultative anaerobes, enterobacteriaceae and streptococci, were detected in the first week of life. They made up the predominant flora for 2 weeks and the numbers of these bacteria decreased to a level similar to those in their mothers in the 3rd week. Their population levels were maintained thereafter. Obligate anaerobic bacteria were detected on the 7th day of life and increased within 2 weeks. They became predominant on the 21st day and thereafter. The intestinal flora of HFA mice were established in their newborn within 3 weeks after birth.

Discussion

The development of intestinal flora of CV mice has been reported [2, 8, 9, 15], and the results obtained in the present study agreed with those of previous studies. On the other hand, the development of intestinal flora of the human infant is different from that of CV mice. In human infants, facultative anaerobes including E. coli and streptococci are detected on the first day of life and are rapidly followed by bacteroidaceae and bifidobacteria. Bifidobacteria become the most predominant bacteria within 1 week after birth [1, 10]. In the present study, bifidobacteria never became the most predominant bacteria in the infants of HFA mice, even though the mothers of HFA mice A harbored large populations of bifidobacteria. Although the composition of intestinal flora of HFA mouse was similar to that of humans, the development of intestinal flora of HFA infant mice did not simulate the development of intestinal flora of human infants.

It has already been reported that GF animals are good recipients for intestinal bacteria of other animal species [4, 5, 14]. However, it was also reported that some bacterial groups, e.g. bifidobacteria and lactobacilli, can colonize in HFA animals but not always [3, 5], and the elimination of such bacteria from HFA animals
seems to be dependent on the composition of flora of inoculated samples [5]. Therefore, it is very difficult to produce HFA mice with the same bacterial flora twice. When we use HFA mice as experimental animals, it is important to maintain human flora in their intestine for a long time. In the present study, intestinal flora of two groups of HFA mice were established in the intestine of HFA infant mice within 3 weeks after birth and the compositions of intestinal flora of both offspring were the same as those of their mothers. These results indicated that HFA mice can be reproduced by breeding.

References