Bacterial Flora of Ex-germfree Mice after Oral Inoculation of Feces from Various Species of Conventional Animals

Kazuyoshi MAEJIMA, Jun SASAKI, Kouji SHIMODA and Tsutomu KUROSAWA*

Laboratory Animal Center, Keio University School of Medicine, Shinjuku, Tokyo 160, Japan, and *Teikyo University School of Medicine, Itabashi, Tokyo 173

(Received for publication : November 11, 1980)

Emulsions of fresh feces from a conventional mouse, rabbit, dog or cynomolgus monkey were administered orally to germfree and conventional ICR mice. Various fecal floras bearing a striking resemblance in pattern to those in the inoculant became established in the inoculated ex-germfree mice. Reinoculation of these ex-germfree mice with fecal emulsions from a conventional mouse made 21 days after the original inoculation (conventionalization) led to alteration of their floras to the indigenous pattern of conventional mice. The administration of feces from the conventional animals of different species did not appreciably modify the fecal flora of conventional mice. The results indicate that intestinal flora of animals is determined in the first stage by that of the contaminants, but this would be altered later under the predominant influence of the various host factors.

The bacterial flora of animals and man is generally thought to be controlled by both the physiological condition of the host and the interaction of microorganisms. There has been, however, much controversy among investigations on this subject though numerous studies have been conducted to determine the factors affecting the flora [1-7]. Recently, Duculzeau et al. [1] successfully transferred the bacterial flora of conventional pigs and piglets to germfree mice, and described that those germfree mice might be of use as an excellent model for studies on the flora of the conventional pig and piglet. Using a technique similar to that described by them, experiments were performed to investigate the establishment and maintenance of intestinal flora in germfree and conventional mice by inoculation of feces from various animal species, enumerating four representative bacteria in specimens. The effect of conventionalization on the ex-germfree mice was also investigated.

Female germfree ICR mice supplied from Teikyo University Laboratory Animal Center and commercially obtained female conventional Slc:ICR mice were used. Mice were kept in isolators sterilized by spraying with 2% peracetic acid and fed commercial pellets irradiated with 5 Mrad of gamma-rays (FR-1, Funabashi Farm). Twenty-four germfree mice were divided into 4 groups, received 0.1 ml of a 10% fecal emulsion prepared from the mouse, rabbit, dog or cynomolgus monkey,
Table 1. Fecal flora of ex-germfree mice after oral inoculation of feces from various animal species

<table>
<thead>
<tr>
<th>Source</th>
<th>Organisms examined</th>
<th>No. organisms in inoculum</th>
<th>Days after administration</th>
<th>1</th>
<th>7</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Enterobacteriaceae</td>
<td>2.99*</td>
<td>7.57±0.89**</td>
<td>5.95±0.43</td>
<td>6.60±0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>2.67</td>
<td>5.30±1.59</td>
<td>5.45±1.10</td>
<td>5.82±1.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>5.95</td>
<td>8.60±0.21</td>
<td>8.57±0.43</td>
<td>8.80±0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>6.04</td>
<td>5.67±1.26</td>
<td>7.75±0.25</td>
<td>8.50±0.39</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Enterobacteriaceae</td>
<td>2.41</td>
<td>≤3.7</td>
<td>≤4.0</td>
<td>≤3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>6.23</td>
<td>8.85±0.54</td>
<td>9.00±0.87</td>
<td>9.55±0.12</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>Enterobacteriaceae</td>
<td>4.02</td>
<td>7.10±0.53</td>
<td>5.45±1.20</td>
<td>6.07±0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>1.45</td>
<td>5.47±0.98</td>
<td>5.70±0.76</td>
<td>5.62±0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>3.88</td>
<td>7.55±1.46</td>
<td>8.55±1.68</td>
<td>7.65±1.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>5.94</td>
<td>9.00±0.45</td>
<td>8.75±0.51</td>
<td>9.05±0.30</td>
<td></td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>Enterobacteriaceae</td>
<td>3.85</td>
<td>7.35±0.53</td>
<td>8.35±0.44</td>
<td>7.12±1.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>4.30</td>
<td>6.17±0.61</td>
<td>8.02±0.86</td>
<td>7.65±0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>6.27</td>
<td>7.55±1.46</td>
<td>8.85±1.68</td>
<td>9.15±1.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>6.25</td>
<td>8.60±0.31</td>
<td>8.85±0.60</td>
<td>9.10±0.77</td>
<td></td>
</tr>
</tbody>
</table>

* log/head
** log/g-feces, Mean± S. D. (N=6)

Table 2. Fecal flora of ex-germfree mice given feces from various animal species 2 days after conventionalization

<table>
<thead>
<tr>
<th>Organisms examined</th>
<th>Enterobacteriaceae</th>
<th>Streptococcus</th>
<th>Lactobacillus</th>
<th>Bacteroides</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. organisms in inoculum</td>
<td>3.10*</td>
<td>3.28</td>
<td>6.06</td>
<td>5.88</td>
</tr>
<tr>
<td>Source</td>
<td>Mouse</td>
<td>6.02±0.35**</td>
<td>5.85±1.05</td>
<td>8.67±0.55</td>
</tr>
<tr>
<td>Rabbit</td>
<td>6.02±0.92</td>
<td>5.67±0.83</td>
<td>8.70±0.29</td>
<td>8.65±0.38</td>
</tr>
<tr>
<td>Dog</td>
<td>6.52±0.55</td>
<td>5.90±1.28</td>
<td>9.00±0.54</td>
<td>8.82±0.23</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>5.80±0.69</td>
<td>6.27±0.97</td>
<td>9.00±0.54</td>
<td>8.47±0.42</td>
</tr>
</tbody>
</table>

*, ** See the foot-notes in Table 1.

respectively, via a stomach tube. Four groups of 6 conventional mice each were treated in the same manner as the germfree mice. To investigate the effect of conventionalization on the exogenous flora of ex-germfree mice, 24 other germfree mice were grouped, treated as in the foregoing series, and then further given orally 0.1 ml of a 10% fecal emulsion obtained from a conventional mouse on the 21st day after the original inoculation.

Feces were carefully collected from
Table 3. Fecal flora of conventional mice after oral administration of feces from various animal species

<table>
<thead>
<tr>
<th>Source</th>
<th>Organisms examined</th>
<th>No. in organisms inoculum</th>
<th>Days after administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Mouse</td>
<td>Enterobacteriaceae</td>
<td>2.99*</td>
<td>5.82±0.45**</td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>2.67</td>
<td>6.07±0.38</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>5.95</td>
<td>8.57±0.17</td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>6.04</td>
<td>8.92±0.68</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Enterobacteriaceae</td>
<td>2.41</td>
<td>5.52±0.35</td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>Neg.</td>
<td>6.12±0.40</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>Neg.</td>
<td>8.75±0.38</td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>6.23</td>
<td>7.50±1.39</td>
</tr>
<tr>
<td>Dog</td>
<td>Enterobacteriaceae</td>
<td>4.02</td>
<td>6.05±0.23</td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>1.45</td>
<td>6.32±0.30</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>3.88</td>
<td>8.60±0.60</td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>5.94</td>
<td>7.35±0.80</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>Enterobacteriaceae</td>
<td>3.85</td>
<td>5.30±1.32</td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>4.30</td>
<td>5.42±1.57</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>6.27</td>
<td>8.82±0.28</td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
<td>6.25</td>
<td>6.90±0.45</td>
</tr>
</tbody>
</table>

* See the foot-notes in Table 1.

The fecal floras of ex-germfree mice following inoculation of fecal emulsions from various animal species are shown in Table 1, along with the bacterial counts of the inocula. Only one day after inoculation, great numbers of bacteria were already found in feces of the inoculated ex-germfree mice. The fecal bacterial floras of these mice had patterns strikingly similar to those of floras in the inoculant in respect of types and population of organisms. In the fecal flora of ex-germfree mice receiving rabbit feces negative for the two genera: Streptococcus and Lactobacillus, neither of these organisms was detectable during the observation period while comparable populations of the other two groups of bacteria were recovered in the fecal flora of these mice. By the 7th day after inoculation, no difference could be detected between the inoculant and the feces of ex-germfree mice. The fecal flora in each group of these mice 28 days after inoculation was still the same as that on the 1st day. These findings suggest that the flora of an inoculant can be
transferred to germfree animals without any appreciable modification, and that the normal indigenous flora in the alimentary tract of conventional animals is generally determined by the flora of the contaminants at birth.

The transferred flora in ex-germfree mice from animals of different species, however, became promptly changed to the normal indigenous pattern in the conventional mice, following inoculation with feces of the conventional one (Table 2). A rapid change of transferred flora in ex-germfree mice after conventionalization was also demonstrated in the previous investigation [5]. As shown in Table 3, the normal flora of mouse was scarcely affected by the administration with exogenous flora. Koopman et al. [3] also found that normal flora resists the colonization of exogenous microorganisms. The results of these experiments show that the various host factors, rather than bacterial interactions, have a predominant influence upon the relative composition of the intestinal flora.

We wish to thank Asist. Prof. T. Ohta, Faculty of Agriculture and Veterinary Medicine, Nihon University for his valuable discussion. We also thank Asist. Prof. H. Tamura, Teikyo University Medical School for supply of germfree mice.

References


各種ふつう動物の糞便を経口投与した
た（元）無菌動物の細菌そう

前島一淑・佐々木淳・下田耕治

慶応義塾大学医学部
*帝京大学医学部

無菌およびふつうマウスにふつうマウス、ウサギ、イス、カニクイザルから採った新鮮な糞便を経口投与したところ、（元）無菌マウスの糞便には、それぞれの投与試料中にみられる細菌構成に似た細菌そうが形成されたが、ふつうマウスにおいては、細菌そうの変化はみられなかった。また、上記（元）無菌マウスにふつうマウスの糞便を投与すると、いずれのマウスの細菌そうもふつうマウスのそれに変った。これらの所見は、動物の消化管細菌そうが宿主の生理条件および微生物の拮抗・共生作用に規定されることを示している。