Comparative Studies of Some Functional and Morphological Parameters in the Livers of Germfree, Conventional and Ex-germfree Mice

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Some parameters of hepatic function and morphology were studied to compare germfree (GF) and conventional (CV) BALB/c mice. The levels of lipid peroxide (LPO) and aniline-hydroxylase (AH) activity in the livers and the serum total cholesterol (TC), triglyceride (TG) and phospholipid (PL) were significantly lower in GF than in CV 8-week-old mice. There were no significant differences in the histology and lectin-histochemistry of the livers in the GF and CV mice. On the other hand, in ex-GF mice which were induced by housing 4-week-old GF mice together with age-matched CV mice, the levels of LPO and AH activity in the liver and the serum TC, TG and PL contents increased rapidly within the first week and then approached values almost identical to those in CV mice 4 weeks later (i.e. at 8 weeks of age). The histologic picture of the liver was similar among the GF, CV and ex-GF mice. KEY WORDS: ex-germfree mouse, germfree mouse, liver

The majority of substances which are absorbed from the intestinal lumen through villous epithelial cells are carried into capillaries in the lamina propria mucosae and reach the liver via the portal vein. Therefore the liver and intestine are very significant organs when we try to compare the biological states in germfree (GF) and conventional (CV) animals. There have been many studies of the biochemical parameters in the livers of GF mice, such as Cyt-P 450 concentration [14], drug metabolizing enzyme activity following drug-treatment [1, 2, 10, 13, 16], and serum glutamate oxaloacetate transaminase activity and total cholesterol content [15]. However, simultaneous biochemical and morphological studies of the livers of GF and ex-GF mice are scarce. This paper describes the changes in some biochemical parameters related to hepatic functions and hepatic morphology in GF, CV and ex-GF BALB/c mice.

Mice were kept in metal cages (22 × 32 × 11 cm) with autoclaved wood chips for bedding in Trexler-type flexible vinyl isolators (60 × 50 × 50 cm; temperature, 23±1°C; humidity, 50±8%). The mice were fed CMF pellets sterilized by 50 KGY gamma-ray irradiation (Oriental Yeast Co. Ltd., Tokyo) and autoclaved water ad libitum. Statistics were analyzed using Student's t-test.

In Experiment 1, 8-week-old GF (10 males) and CV mice (10 males) were examined. As shown in Fig. 1, the liver-to-body weight ratio was lower in GF than in CV mice (p<0.001), though the body weight was similar in the two groups. The lipid peroxide (LPO) content in the liver, expressed as the malondialdehyde (MDA) content by the TBA method [17], was lower in GF than in CV mice (p<0.001) (Fig. 1). This may reflect the amount of foreign substances with liver-stimulating effects which were transported from the intestine to the
liver. It is said that LPO is easily produced from unsaturated fatty acids during the process of detoxication of foreign materials in the liver [7, 12].

There are many reports that GF animals have a low capacity to metabolize certain drugs [1, 3, 10, 13, 16]. The present results showed that aniline-hydroxylase (AH) activity measured by the method of Nash [9] was lower in GF than in CV mice (p<0.001), while there was no significant difference in aminopyrine-N-demethylase (ADM) activity measured by the method of Imai et al. [4] between GF and CV mice (Fig. 1). This indicates that the ADM activity was less influenced by GF or CV status than the AH activity.

Serum biochemical analysis by colorimetry using an auto-analyzer (Monarch, Instrumentation Laboratory, USA) showed that the levels of phospholipid (PL), triglyceride (TG) and total cholesterol (TC) were lower in GF than in CV mice (p<0.001), but there were no significant differences in the levels of total protein (TP), albumin (AL) or blood urea nitrogen (BUN) in the GF and CV mice (Table 1). The results were different from the previous reports on rats showing significant differences in BUN and TP but not in TG and TC between GF and CV rats [6, 11, 15]. Moreover, the results were contrary to the report by Watanuki et al. showing a lowering effect of intestinal microflora on serum lipid concentrations in rats due to reduction in the lipid-absorption rate through the intestinal mucosa [18]. The reason for such discrepancy between rats and mice is obscure.

Contrary to our expectation, there was no significant difference in the liver histology between GF and CV mice. There have been no previous reports on the lectin-histochemistry of the liver of GF mice, but the present study using 10 lectins (E-Y Laboratories, San Mateo, CA) revealed that the lectin-binding activities in the liver of GF and CV mice were identical. The lectin-binding activities in the liver of the CV mice used in the present study were similar to those reported in CV mice of other strains [5]. Morphometrical studies by electron microscope are now in progress.

In Experiment 2, 15 male GF mice which were housed together with 15 age-matched 4-week-old male CV mice were used as ex-GF mice. Five ex-GF mice were killed 1, 2 and 4 weeks later (i.e. at 5, 6 and 8 weeks of age) and 5 CV mice were sacrificed 0, 2 and 4 weeks later (i.e. 4, 6 and 8 weeks of age). In addition, 5 male GF mice were killed at 4 and 8 weeks of age, respectively.

As observed in the 8-week-old mice, 4-week-old GF mice showed lower levels of liver-to-body weight ratios and lower LPO content and AH activity in the liver than age-matched CV mice (p<0.001). These parameters in ex-GF mice increased rapidly within the first week and then approached levels not significantly different from those in CV mice 4 weeks later (i.e. 8 weeks of age) (Fig. 2). This was probably due to a rapid increase in the amount of foreign substances with liver-stimulating effects in the portal vein following colonization by intestinal microflora. Koopman et al. [8] have reported that the colonization by intestinal microflora occurred in ex-GF mice within 5 days.

The levels of serum PL, TG and TC in GF mice were similar to those in 4-week-old CV mice. Thereafter, the levels of these parameters in the CV mice were elevated gradually with time. But in ex-GF mice, they became rapidly elevated within the first week and then approached those in CV mice 4 weeks later (i.e. at 8 weeks of age) (Fig. 3).
Table 1. Serum biochemistry in 8-week-old germfree (GF) and conventional (CV) mice

<table>
<thead>
<tr>
<th></th>
<th>TP (g/dl)</th>
<th>AL (g/dl)</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>PL (mg/dl)</th>
<th>BUN (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>GF</td>
<td>4.7 ± 0.4</td>
<td>3.2 ± 0.3</td>
<td>156*</td>
<td>93*</td>
<td>210*</td>
<td>23.8</td>
</tr>
<tr>
<td>CV</td>
<td>5.5 ± 0.6</td>
<td>3.8 ± 0.3</td>
<td>255</td>
<td>134</td>
<td>286</td>
<td>27.7</td>
</tr>
</tbody>
</table>

Each value indicates the mean ± SD of 10 mice. * Statistically different from CV mice (p<0.001). TP: total protein AL: albumin TG: triglyceride TC: total cholesterol PL: phospholipid BUN: blood urea nitrogen

Fig. 2. Changes in the liver-to-body weight ratio (A), lipid peroxide content (B) and aniline-hydroxylase activity (C) in germfree (●), conventional (○) and ex-germfree mice (△). Each value indicates the mean ± SD of 5 mice. *Statistically different from CV mice (p<0.001)

Fig. 3. Changes of serum phospholipid (A), triglyceride (B) and total cholesterol (C) in germfree (●), conventional (○) and ex-germfree mice (△). Each value indicates the mean ± SD of 5 mice. *Statistically different from CV mice (p<0.001)
In the histological findings, there was no apparent difference in the livers of GF, CV and ex-GF mice from 4 to 8 weeks of age. In conclusion, some biochemical parameters related to hepatic functions showed significant differences between GF and CV mice. The values of such parameters in ex-GF mice increased rapidly and were similar to those in CV mice at 8 weeks of age. In contrast, there was no significant difference in histology and lectin-binding activity of the liver among the GF, CV and ex-GF mice.

References