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ABRUPT INDUCTION OF GDP-FUCOSE: ASIALO GM1 FUCOSYLTRANSFERASE IN THE SMALL INTESTINE AFTER CONVENTIONALIZATION OF GERM-FREE MICE

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We studied the time-course of the induction of GDP-fucose: asialo GM1 fucosyltransferase and its product, i.e. fucosyl asialo GM1, of the small intestine after introduction of microorganisms to germ-free mice (conventionalization). We found that the fucosyltransferase activity was abruptly induced and asialo GM1 was converted into fucosyl asialo GM1 within a few days after conventionalization. However, two weeks after conventionalization this enzyme activity dropped to approximately 10^{-2} level of the maximum value and asialo GM1 appeared again as one of the major glycolipids. These results showed that the microbial colonization in the gut evoked a drastic change of the glycolipid pattern at the intestinal epithelial cell-surface via the induction of a fucosyltransferase.

The presence of microflora in the alimentary tract is thought to play an important role for intestinal transport (1) and development of the immune system (2,3). Association with microorganisms to germ-free animals, on the other hand, alters the intestinal structures, e.g. villous height and crypt-depth (4,5). However, there is little biochemical approach to these problems. Comparing the structure and biosynthesis of the glycolipids of the intestinal epithelial cells between germ-free and conventionalized ex-germ-free mice. We found that the localization of fucosyl asialo GM1 was restricted to the microvillus membrane of conventionalized mice (6,7). The amount of fucosyl asialo GM1 4 weeks after conventionalization was much smaller than that of the other major glycolipids, i.e. asialo GM1 and glucosylceramide (6). In this article, we report the precise time-course of GDP-fucose: asialo GM1 fucosyltransferase induction and dramatic change of the glycolipid pattern in the small intestinal epithelial cells after conventionalization of germ-free mice.

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MATERIALS AND METHODS

Germ-free ICR female mice (8-week old) were randomly divided into two groups. One group was maintained in a flexible vinyl isolator under standard germ-free conditions (8). Another group were removed from the isolator and orally administered the suspension of fresh stool of conventional ICR mice. These mice were reared in a conventional animal room. Both groups of mice received the sterilized diet and water ad libitum. The temperature, humidity and lighting-schedule were identical in both groups.

The enzyme solution for GDP-fucose: asialo GM1 fucosytransferase was prepared from oral halves of the small intestines of decapitated mice. After washing with cold saline, mucosa was scraped with smooth edged glass slide, suspended in 50 mM cacodylate-HCl buffer (pH 5.8) and stored under -20°C. On the day for the enzyme assay frozen samples were melted and homogenized with Potter-type homogenizer (enzyme source). The enzyme activity was stable at -20°C for at least one month. We adopted the assay method of Taki et al. (9) with some modifications. Reaction mixture contained 0.37 μmole asialo GM1 from the mouse small intestine, 20 mM $MnCl_2$, 1% Triton X-100, 5 nmol GDP-[1-^{14}C]-fucose (20 mCi/mmol), 0.4 mg enzyme protein, and 50 mM cacodylate-HCl buffer (pH 5.8)(total volume 100 μl). Asialo GM1 was added as chloroform-methanol (2:1, by vol.) solution. After evaporation, asialo GM1 was sonified in the buffer using water-bath type sonicator. Enzyme reaction was done at 30 °C with shaking and was terminated by the addition of 5 volumes of chloroform-methanol (2:1, by vol.). Resultant upper phase was partitioned again with theoretical Folch's lower phase solvent (10). Pooled lower phase solution was evaporated under N_2 stream. 90.4±2.4 (S.D.)% of fucosyl asialo GM1 was quantitatively recovered in the residue. This residue was applied to TLC (precoated silica gel plastic sheet, Merck) and developed in the solvent system of chloroform-methanol-water (60:40:4, by vol.). The area of fucosyl asialo-GM1 was cut off by scissors and the radioactivity was counted in toluene scitillator using a liquid scintillation counter.

Mucosal homogenate of 7.0 mg protein were extracted with 20 volumes of chloroform-methanol (2:1) for 6 hr by end-over-end shaking and filtrated on a filtration paper. The residue was washed with 10 volumes of chloroform-methanol (1:1, by vol.). After evaporation of pooled filtrate to dryness, the residue was acetylated. This was applied to an iatrobeads column chromatography (2). Asialo GM1 and fucosyl asialo-GM1 was eluted with acetone. After deacetylation and neutralization of the acetone fraction (2), this was applied to TLC (precoated HPTLC, Merck) and developed in the solvent system of chloroform-methanol-water (60:40:8). Glycolipids were visualized by spraying of 50% H_2SO_4 and heating to 110°C.

The content of protein and sugar were determined by the method of Lowry et al. (11) and phenol-sulfuric acid method (12), respectively.

RESULTS AND DISCUSSION

We determined the assay conditions for GDP-fucose: asialo GM1 fucosayltransferase of the mouse small intestinal epithelial cells based on the methods for rat ascites hepatoma cells (6). We preferred 30°C as the assay temperature due to high nucleotide pyrophosphatase in the intestinal epithelial cells (13). Triton X-100 was the only effective detergent among tested. The optimum pH was 5.8 as in rat ascites hepatoma cells (9). The most effective concentration

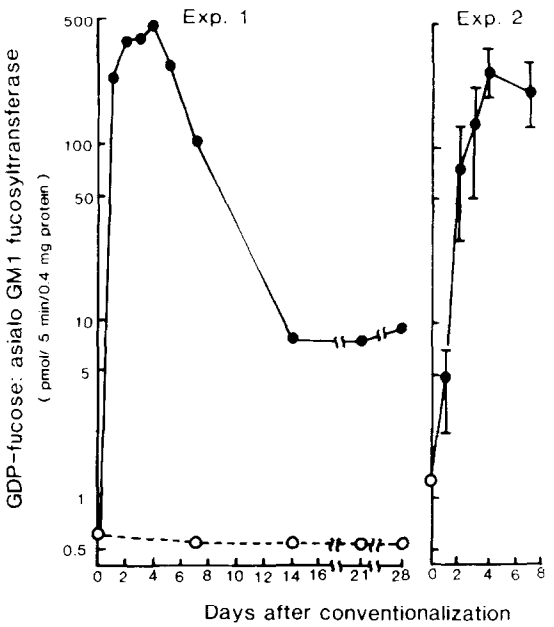

Fig. 1 Time-course of GDP-fucose: asialo GM1 fucosyltransferase induction after conventionalization of germ-free mice. Germ-free mice were conventionalized at the age of 8 weeks. Methods for preparation of the enzyme source and enzyme assay were described in the text. Each point of Exp. 1 and Exp. 2 represents an average value for two and three (± S. E.) mice, respectively. Open symbols show the control value obtained from the mice keeping in germ-free conditions.

of $MnCl_2$ and Triton X-100 were 20 mM and 1.0%, respectively. The activity was linear up to 0.7 mg enzyme protein and up to 15 min. In the case of high activity, the reaction was shortened to keep the linearity.

Fig. 1 shows an abrupt increase in the fucosyltransferase activity within a few days after conventionalization of germ-free mice. Two weeks after conventionalization, the enzyme activity dropped by approximately 10^{-2} from the maximum value. The activity in germ-free mice was negligible throughout the experimental period from 8 to 12 weeks old. Moreover, we examined the product of this enzyme reaction. The glycolipids were extracted from the mucosal homogenate which were sampled at different days after conventionalization and applied to TLC (Fig. 2). As expected asialo GM1 was, if not all, converted to fucosyl asialo GM1 within a few days after conventionalization. Although fucolipid was not detectable on TLC 14 or 28 days after conventionalization, small amount (approximately 5% of asialo GM1) of fucosyl asialo GM1 existed as

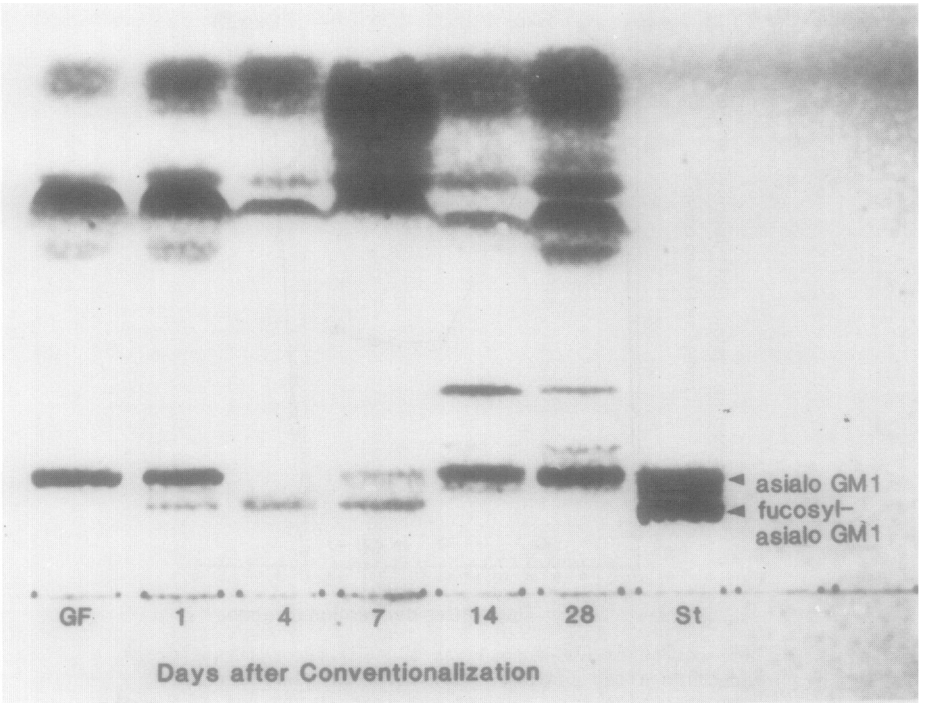

Fig. 2 A thin layer chromatogram of the lipid fractions containing asialo GM1 and fucosyl asialo GM1. At the days indicated after conventionalization, glycolipids fractions were prepared from the mucosal homogenate (7.0 mg protein) as described in the text. TLC was developed with chloroform-methanol-water (60-40-8, by vol.) and glycolipids were detected by spraying with 50% H_2SO_4. Lane (St) indicates the standard glycolipids isolated from mouse small intestine (7).

previously reported (1). A preliminary structural analysis of fucosyl asialo GM1 showed that fucose was attached to the terminal galactose of asialo GM1 with α(1-2) linkage (unpublished data).

We have not yet confirmed the absence of this enzyme protein in germ-free mice. The presence of enzyme inhibitors in the enzyme sources of germ-free mice or the presence of enzyme activators in those of conventionalized mice are, however, unlikely because the mixture of the enzyme sources from germ-free and conventionalized mice showed stiocheometrical activity dependent solely on the protein concentration of conventionalized mice.

A monocontamination with Bacteroides sp. or Fusobacterium sp., which are dominant microbes in the lower gut (14), to germ-free mice could not induce the enzyme activity at all (data not shown). In the first week after conventionalization, drastic change of population of the microorganisms occurs to

establish a steady microflora in the gut (5, 15). Therefore, this change of microbial population shortly after conventionalization may hold an important key of the fucosyltransferase induction, though it is not easy to elucidate what stimuli are produced by microbial association.

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