Epithelial-mesenchymal interaction in differentiation of duodenal epithelium of fetal rats in organ culture[1]

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Summary. Epithelial-mesenchymal interaction in the differentiation of duodenal epithelium of fetal rats was investigated by recombination experiments in vitro. The proportion of goblet cells in duodenal epithelium was significantly greater on recombination of developing duodenal epithelium with mesenchyme of the glandular stomach than on recombination with that of the duodenum. Mesenchyme of the glandular stomach or forestomach was better than duodenal mesenchyme in supporting morphogenesis of duodenal epithelium. Treatment of tissues with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) did not affect these tissue interactions.

Epithelial-mesenchymal interactions in the differentiation of the epithelium of the gastro-intestinal tract has been investigated by many workers. We have demonstrated that in fetal mice the developmental fate of the stomach epithelium is not affected by recombination with heterologous mesenchymes on and after day 11.5 of gestation, but that the expression of epithelial morphogenesis and cytodifferentiation is still under the influence of the mesenchyme in prenatal stages[3]. We have also reported that epithelial keratinization is promoted by treatment of fetal rat forestomach with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in organ culture[4]. In this work we examine whether differentiation of duodenal epithelium is affected by recombination with heterologous mesenchyme or treatment with MNNG.

Materials and methods. Tissue fragments of the forestomach, glandular stomach and duodenum were obtained from 16.5-day-old fetuses of inbred Fischer 344/DuCrj rats (Charles River Japan, Inc.). They were treated with 5 µg/ml of MNNG (Aldrich Chemical Co.) for 1 h at 37 °C in the dark, washed thoroughly with Hanks' solution, and then soaked in a solution of collagenase (Worthington Biochemical Co.) for 90 min at 37 °C. The epithelium and mesenchyme were separated with forceps and, after inactivating the enzyme by washing the tissues with Hanks' solution containing serum, the duodenal epithelium was recombined with the mesenchyme of the forestomach, glandular stomach or duodenum. Recombinants were cultivated on membrane filters (Millipore Corp.) laid on stainless steel grids placed in dishes. The culture medium was medium 199 (Grand Island Biological Co.) supplemented with 10% fetal bovine serum (Microbiological Associates). The recombinants were cultivated in a humidified atmosphere of 5% CO_2 in air for 7 days without medium change. Then they were fixed with Bouin's fluid, embedded in paraffin, sectioned serially at 5 µm and stained with PAS-Hematoxylin. The number of goblet cells, identified by PAS-positive droplets, was counted in every 20th section, and the proportion of goblet cells among the total epithelial cells was calculated as the average of the proportions in 3–5 sections

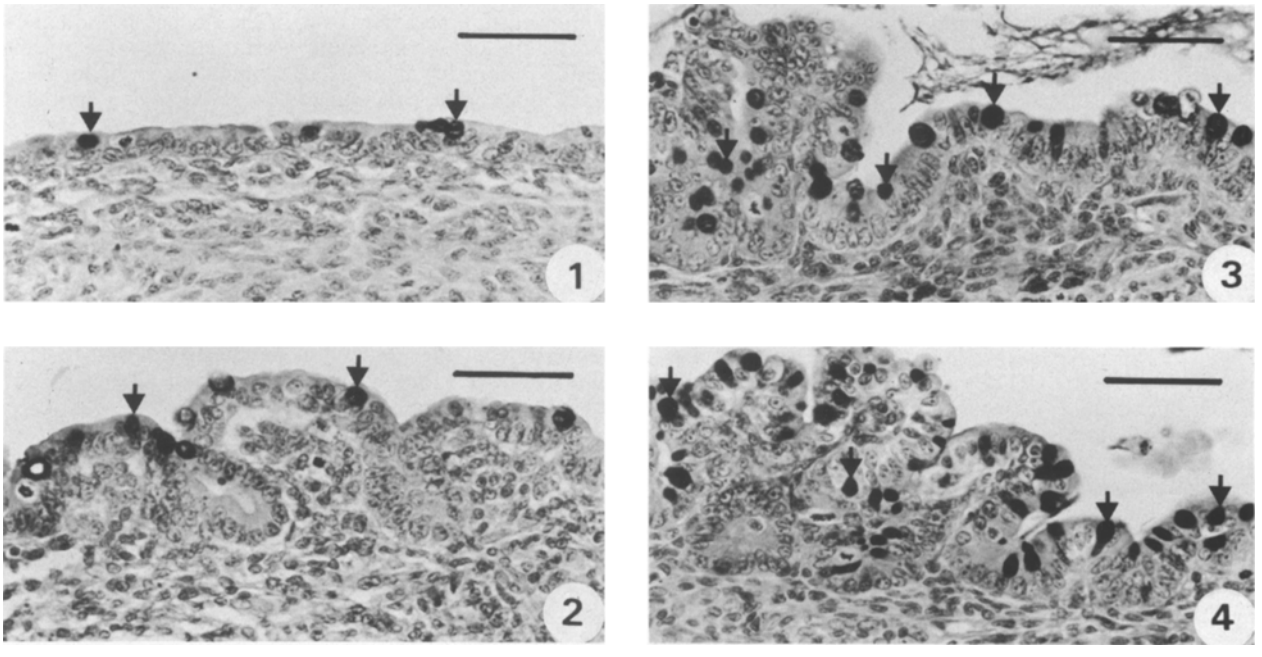

Fig. 1. Recombination of control duodenal epithelium and control duodenal mesenchyme. Simple columnar epithelium without gland structures, but with a few goblet cells (arrows) is seen. The bar represents 50 µm. × 300.

Fig. 2. Recombination of MNNG-treated duodenal epithelium and MNNG-treated duodenal mesenchyme. Simple columnar epithelium with gland structures and a few goblet cells (arrows) is seen. The bar represents 50 µm. × 300.

Fig. 3. Recombination of control duodenal epithelium and control mesenchyme of the glandular stomach. The epithelium forms villi with many goblet cells (arrows). The bar represents 50 µm. × 300.

Fig. 4. Recombination of MNNG-treated duodenal epithelium with MNNG-treated mesenchyme of the glandular stomach. Simple columnar epithelium with gland structures and many goblet cells (arrows) is seen. The bar represents 50 µm. × 300.

Table 1. Percentage of goblet cells in duodenal epithelium cultured after recombination with mesenchyme from various regions of the digestive tract

Mesenchyme		Epithelium = duodenum	
Origin	Treatment	MNNG-treated	Control
Forestomach	MNNG-treated	10.1±4.4 (6)	5.8±4.8 (12)[c]
	Control	8.7±3.8 (9)	8.4±2.8 (11)
Glandular stomach	MNNG-treated	14.2±4.4 (7)[a]	11.8±3.8 (7)[c,d]
	Control	11.2±4.1 (7)[b]	10.7±1.7 (7)[e]
Duodenum	MNNG-treated	6.8±2.9 (4)[a]	6.7±3.5 (6)[d]
	Control	6.8±2.7 (6)[b]	6.7±2.3 (9)[e]

Mean±SD (number of samples). Student's t-test; [a] $p<0.05$, [b] $p<0.05$, [c] $p<0.02$, [d] $p<0.05$, [e] $p<0.01$.

Table 2. Rate of formation of epithelium with gland structures on recombination with mesenchyme from various regions of the digestive tract

Mesenchyme		Epithelium = duodenum		
Origin	Treatment	MNNG-treated	Control	Total[a]
Forestomach	MNNG-treated	5/6	10/12	34/38[b]
	Control	9/9	10/11	
Glandular stomach	MNNG-treated	7/7	7/7	28/28[c]
	Control	7/7	7/7	
Duodenum	MNNG-treated	1/5	2/6	14/25[b,c]
	Control	6/6	5/9	

[a] On the assumption that MNNG-treatment had no effect. χ^2-test; [b] $p<0.01$, [c] $p<0.001$.

in 1 sample. The results were analyzed statistically by the Student's t-test or the χ^2-test, and differences were considered significant when $p<0.05$.

Results. Undifferentiated stratified epithelium of 16.5-day-old fetal rat duodenum differentiated into simple columnar epithelium with goblet cells, and in many of the samples it formed glands or pits. The developmental fate of the duodenal epithelium was not affected by heterologous mesenchyme.

Some of the recombinants are shown in figures 1–4, and the proportion of goblet cells among the duodenal epithelial cells is summarized in table 1. The proportion was significantly higher on recombination with mesenchyme of the glandular stomach than on recombination with mesenchyme of the duodenum. Treatment of the tissues with MNNG tended to increase the proportion in recombinants with heterologous mesenchyme, but had no effect in those with homologous mesenchyme.

The effect of mesenchyme on the formation of gland structures in the duodenal epithelium is summarized in table 2. Glands and pits were formed in all recombinants with heterologous mesenchyme, but only in some recombinants with homologous mesenchyme. Treatments with MNNG did not seem to affect epithelial morphogenesis.

Discussion. Epithelial-mesenchymal interaction in determination of the digestive tract epithelium in mammalian fetuses has been investigated by only a few workers[3,5,6]. The present work showed that the recombination with mesenchyme did not affect the developmental fate of the epithelium, but did affect its expression. These findings are consistent with previous reports that the fate of the epithelium of the fetal mouse stomach is determined by day 11.5 of gestation[3], because the developmental stage at day 16.5 in rat fetuses corresponds to that at day 14.5 in mouse fetuses[7,8].

The proportion of goblet cells in the duodenal epithelium was higher on recombination with mesenchyme of the glandular stomach than on recombination with duodenal mesenchyme. The function of the epithelium is probably greater when there are fewer goblet cells, because almost all epithelial cells in the duodenum other than goblet cells are absorptive. Thus the present results may be interpreted as indicating that homologous mesenchyme keeps the epithelium functional.

Morphogenesis of the duodenal epithelium was supported better by heterologous mesenchyme than by homologous mesenchyme. This indicates that the mesenchyme acts only permissively on epithelial morphogenesis once the developmental fate of the epithelium has been determined. The same conclusion has been obtained from studies on the fetal mouse stomach[3].

Treatment with MNNG did not affect the proportion of goblet cells in the duodenal epithelium appreciably, although it promoted epithelial keratinization in the fetal rat forestomach. Some hormones, such as thyroxine and hydrocortisone, have been reported to affect the proportion of goblet cells in the epithelium[9]. Our system should be useful in investigating the mechanism of differentiation of duodenal epithelium in organ cultures.

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