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Influence of EGF on Gut Development in Mice

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The stimulation of enterocytes with growth factors in organ culture have great importance for the structural and functional study of the gut mucosa and could ensure understanding of the basic aspects of intestinal epithelium cells differentiation in early stages of postnatal development. We studied enzyme activity of lactase and alkaline phosphatase and carried out electron microscopy studies of enterocytes of newborn mice. We established that EGF promotes morphological and functional development of the murine small intestine and affects the growth and development of gastrointestinal tract.

Key words: small intestine, SEM, EGF, enzymes.

Introduction

Human and mammals colostrums contain bioactive substances called "growth modulators" [1]. The last include growth factors, some of which can directly influence the newborn metabolism after gut absorption and promote the growth and differentiation of different tissues [2].

One of the most prominent growth factors in colostrum, by means of quantity is EGF. It is considered in the literature as the main activator in human milk, which stimulates cell division and migration, induces gene expression of mucus enzymes and different peptides, stimulates gut regeneration at necrotizing entherocolitis and mucus inflammation, regulates enzyme activity.

The aim of the presence study was to determine the influence of the EGF on small intestinal development in murine organ culture by means of enterocytes morphology and alkaline phosphatase and lactase enzyme activity.

Materials and Methods

Organ culture: The newborn mice Balb/c were used to isolate the small intestine. The explants were cultivated from 24h to 96h in culture medium RPMI 1640 containing 10% fetal bovine serum at 37°C, 5% CO_2 and 100% humidity. We added 50 ng/ml EGF per well, except for the control specimen, which were cultivated without growth factor.

Scanning electron microscopy: The specimen were fixed in 2.5% glutaraldehyde containing 0.4M Na-caccodylate. The explants were post-fixed in 1% osmium tetraoxide. The specimens were prepared for observation with scanning electron microscope (JEOL JSM 35).

Enzyme histochemistry: The specimen were embedded in tissue freezing medium and were cryo cut to obtain 10 μm thin sections. We prepared substrate media for two enzymes:

Alkaline phosphatase activity: The substrate medium consisted of 0.5mM menadiol diphosphate disodium salt (substrate), 1 μg/ml NBT (nitroblue tetrazolium) and 0.005mM methoxyphenazine methasulfate in 0.1M Tris/HCl, pH 9.2. The incubation lasted 15 min at 37°C.

Lactase activity: The substrate medium consisted 0.5mM 5-bromo-4-chloro-1-indoline-β-D-galaktopyranoside (substrate), 1mg/ml NBT, 0.005mM methoxyphenazine methasulfate in 0.1M Citrate buffer, pH 6.0. The incubation lasted 2 h at 37°C.

Results

Scanning electron microscopy: Characteristic for the early stage of the morphogenesis of intestinal villi is their finger-like shape, upright to the gut lumen. The cells are typically polygonal like hive facets (Fig.1), they are arranged tightly to each other like pavement, and only in some spots loose cell junctions could be observed. The microvilli are well shaped and cover entire striated apical cell surface.

In control samples the enterocytes are not well distinguished, their polygonal shaped is not well structured and cell junctions cannot be well observed (Fig. 2).

Enzyme activity examination: *Alkaline phosphatase* is a marker for differentiated intestinal villous cells and usually is localized at the apical membrane of the cells *in vivo*.

We have observed that in duodenum explants cultivated for 96h have enterocytes, which gave reaction for presence of alkaline phosphatase (Fig. 3). In the figure it makes an impression that the enzyme is localized predominantly on the top of the villi and the cells are more poorly stained in the crypt direction. This effect is connected with cell differentiation stage.

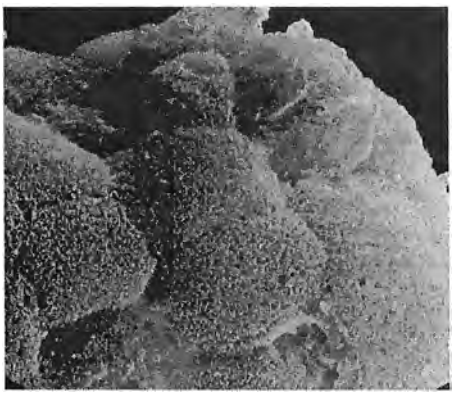

Fig. 1. Explants from duodenum, cultivated with EGF for 72h. Originally × 3900

Fig. 2. Explants from duodenum, cultivated without EGF for 72h. Originally × 3900

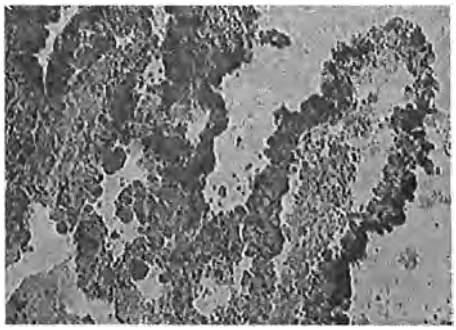

Fig. 3. Alkaline phosphatase: duodenum cultivated with colostrum for 96h. Originally × 100

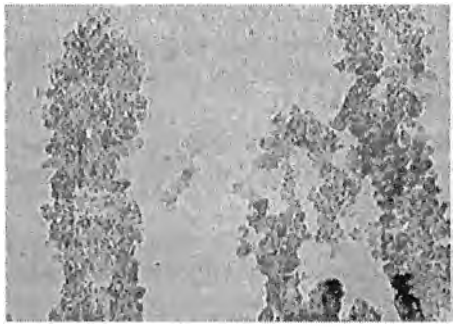

Fig. 4. Alkaline phosphatase: duodenum cultivated without colostrum for 96h. Originally × 100

Fig. 5. Lactase: Jejunum cultivated and stimulated with EGF for 72h. Originally × 400

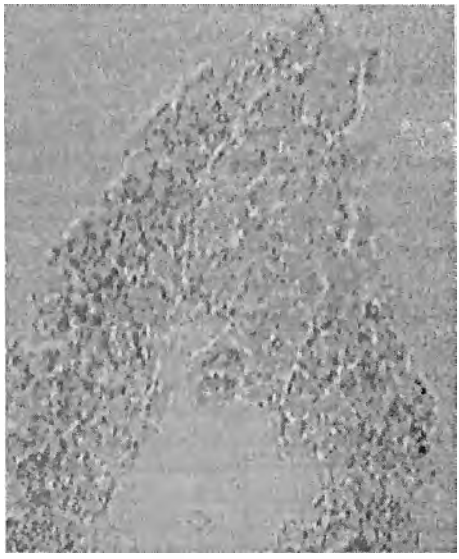

Fig. 6. Lactase: Jejunum cultivated and stimulated without EGF for 72h. Originally × 400

The control samples are slightly stained which is distinctive feature for lower enzyme activity. This trend is also observed in the explants from jejunum and ileum cultivated 48-72 h respectively. In control samples only separate cells display presence of enzyme (Fig. 4).

Lactase: in explants from duodenum and jejunum, cultivated for 48-72 h respectively (Fig. 5), reveal well expressed enzyme activity in contrast to controls (Fig. 6), in which single cells show enzyme presence.

The maturation stage in newborn gut is related to changes in lactase activities and the latest is determined solely by gut mucosal growth and differentiation changes. The diminishing in lactase absorption could play a crucial role in development of food intolerance, effect that occurs frequently in premature infants.

Discussion

The results of the present study indicate that EGFR-signalling has an important function in regulating the newborn gut crypt/villous axis and goblet cell maturation during the organ culture. The newborn mouse gut in organ culture showed many developmental similarities to those grown *in vivo*. Early intestinal villous development *in vitro* indicates an anterior-posterior pattern of regionally specific epithelial differentiation. Moreover the clustering of proliferative epithelial cells to intervillous crypt region in the proximal small intestine during organ culture stimulates normal gut maturation in which the intestinal epithelium organizes into proliferative crypt region and terminal differentiated villi with characteristic for them enzymes.

We have shown that exogenous EGF produces a complex pattern of *in vitro* growth effect on newborn mouse gut. The effects are regionally distinct as EGF 50 ng/ml affect small intestinal growth. Although we have not directly demonstrated how exogenous EGF has access to its cognate receptor on the intestinal epithelium, the observed biologic effects of exogenous EGF on newborn mouse gut growth and development indicate that significant receptor-ligand interaction had occurred [3].

The EGFR-signalling system regulate the growth and maturation of the embryonal and newborn small intestine to crypt-villous axis. This mechanism has potential application in necrotizing entherocolitis therapy that affect severely premature infant [4].

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