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Developmental Study of Murine Gut Mucosa by Scanning Electron Microscopy

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The morphology of mucosa of terminal ileum during postnatal development of mice has been investigated using scanning electron microscopy. The age-related changes in small intestinal mucosa in mice of different age (newborn, 5, 15, 25 and 29 days old) were shown and analyzed. The results demonstrated marked differences in morphological parameters as height and shape of small intestinal villi between investigated periods. This study is the first detailed SEM examination of morphology of developing murine gut and is a part of work aimed to elucidate the role of mother's milk in maturation of the gastrointestinal tract of the offspring in mammals.

Key words: development, mucosa, small intestine, ileum, villi, scanning electron microscopy.

Introduction

The development of mammalian gut after birth is an important physiological phenomenon that is determined and modulated by a complex of factors: genetic developmental programming factors, suckling, weaning, food composition, microbial flora, hormones and growth factors [1]. Despite of many experimental data concerning the postnatal gut development in mammals, knowledge about this process regarding morphological changes in the small intestinal architecture during ontogenesis is still controversial and incomplete.

This study was designed to examine the maturational changes of small intestinal mucosa during the first 30 days of postnatal life in mice using scanning electron microscopy.

Material and Methods

Balb/c mice aged 0,/newborn/, 5, 15, 25 and 29 days were used. Tissue sections (1cm) were taken 0,5 cm proximal the ileocaecal junction. Tissue preparation for SEM was performed by the method reported by P o t t e r et al. [4] with some modifications.

Briefly, the tissue specimens were fixed with 2% glutaraldehyde in 0,05M phosphate buffer (pH 7,4) for 48h at 4°C, followed by postfixation (1% OsO_4 in 0,1M phosphate buffer with added 0,15M sucrose) for 45 min at 4°C. They were subsequently dehydrated, dried in a critical point drier and coated with gold. The specimens were examined with scanning electron microscope (JEOL, JEM-35) under five magnifications: × 100, × 360, × 860, × 4000 and × 10 000.

Results and Discussion

We found significant changes in the morphology of small intestinal mucosa during the postnatal development of murine gut. A gradual increase in villous height was observed between the investigated postnatal periods up to day15. (Fig. 1 — *A*, *B*, *C* and *D*). Using higher magnification of the same objects, the morphologic features of the luminal surface of small intestine, mentioned above, could be more clearly demonstrated (Fig. 2 — *A* and *B*). Our findings confirmed the previous results obtained by other morphological methods that there is an increase of villous length during the first 3 weeks of postnatal life in mice [2], and also that the major changes of gut morphology are associated with the weaning of mammals [1].

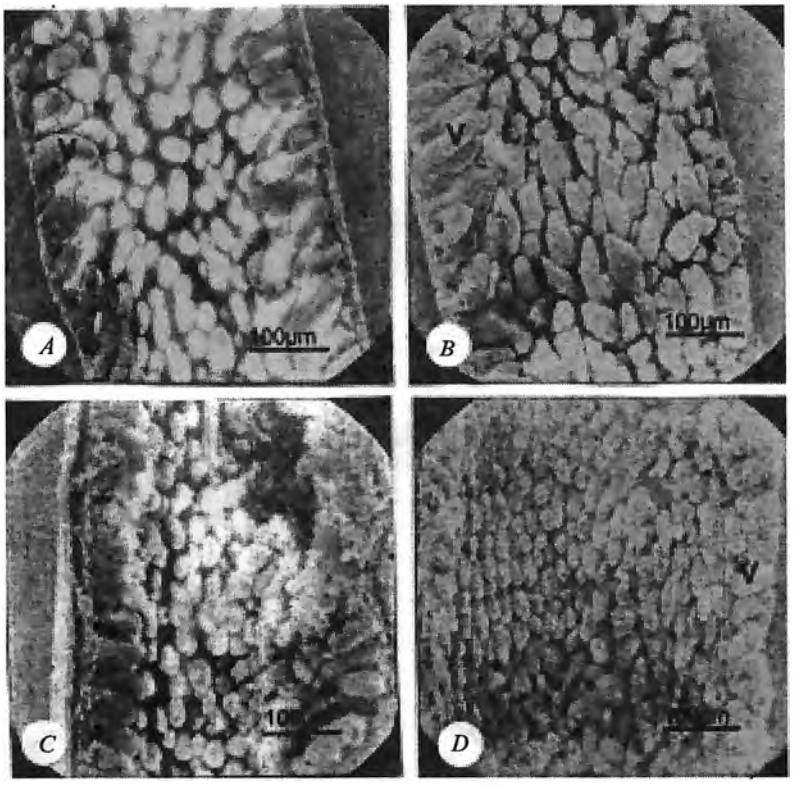

Fig. 1. Scanning electron micrographs of ileal small intestinal mucosa of mice of different age: newborn (A), 5-day-old(B), 15-day-old(C) and 25-day-old(D) mice. V — villi. Orginal magnification ×100, bar 100µm

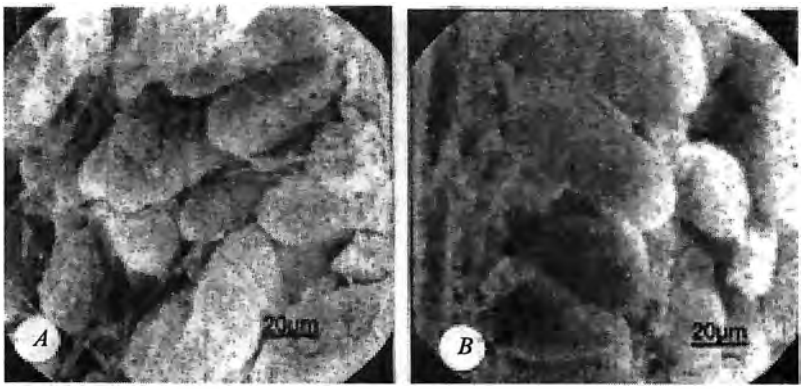

Fig. 2. Scanning electron micrographs of the same tissue samples from 5(A) and 15-day-old(B) mice at highermagnification. Orginal magnification ×360, bar 20μm

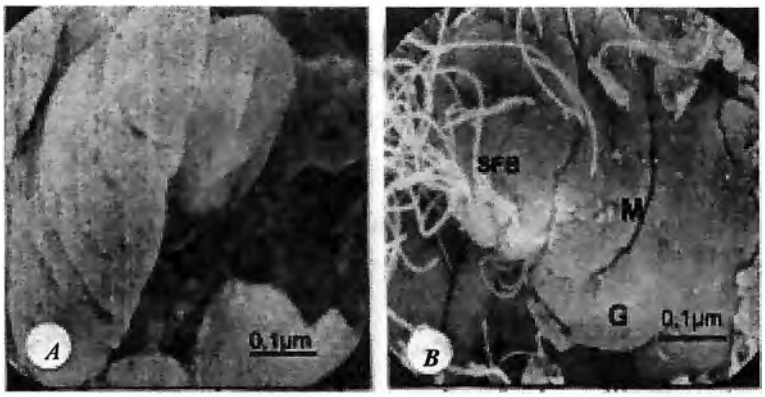

Fig. 3. Scanning electron micrographs represent morphological details of villous surface. A: 5-day-old; B: 29-day-old mice. Orginal magnification ×860, bar 0,1μm. G — goblet cell orifices, M —mucus

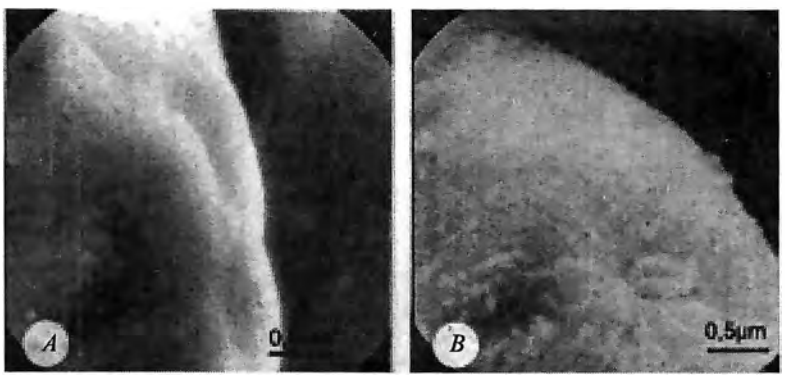

Fig. 4. Scanning electron micrographs of microvillous surfaces of newborn (A) and 15-day-old (B) mice. Orginal magnification ×4000, bar 0,5μm

Another aspect of villous morphology was the dynamics of villous shape. As shown in figures, at birth the villi are predominantly finger-like (Fig. 1 — *A*]. In the next age periods investigated leaf- and ridge-shaped villi dominated (Fig. 1 — *B*, *C* and *D*). We suggest that the shape of the villi could be utilized as a marker for maturational stage of gut mucosa.

An interesting finding was the difference in villi pattern among the investigated periods. At birth (day 0) the villi of the small intestinal mucosa had irregular pattern (Fig. 1 — *A*). Villi either with normal shape and size (high and thin/or smaller villi short and thick), could be distinguished. Our findings illustrated an early stage of maturation of the small intestinal mucosa which is characterised morphologically with irregular villous development: smaller villi were not enough developed as spatial structure. By day 5 villi became more uniformed (Fig. 1 — *B*) and at day 15 villi reached their maximum uniformity in height (Fig. 1 — *C*). This gradual change from irregularity to uniformity in villi height was the major feature of the morphology of developing mouse ileum.

Discrete details of villous morphology could be revealed under higher magnifications [3]. The results demonstrated that the corrugations of the villous surface were obvious in all investigated age periods. The orifices of Goblet cells without or with mucous secretions were well visible (Fig. 3 — *A* and *B*). Presence of segmented filamentous bacteria (SFB) in small intestine of 29-day-old animal (Fig. 3 — *B*) was an interesting finding. Finally, at instrumental magnification ×4000 the apical surfaces of enterocytes building villi could be observed (Fig. 4). In newborn mice microvillous surface was not well developed (Fig. 1 — *A*). The well differentiated microvillous surface were present on day15 and progressively developed up to day29 of age (data not shown).

The results presented here demonstrate marked age related dynamics of morphology of small intestinal mucosa in Balb/c mice. Information gained in this study will help to better understand the process of gut mucosa development during postnatal life.

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