Species-Differences in the Process of Apoptosis in Epithelial Cells of the Small Intestine: An Ultrastructural and Cytochemical Study of Luminal Cell Elements*

Hongxia HAN, Toshihiko IWANAGA and Tsuneo FUJITA

Department of Anatomy, Niigata University School of Medicine, Niigata, Japan

Received January 16, 1993

Summary. Our previous study demonstrated that in the small intestine of guinea pigs, apoptotic epithelial cells at the villus tips were phagocytosed by lamina propria macrophages, leaving only apical cytoplasmic plates, which thereafter were domed and extruded into the lumen. This finding contrasts with the generally accepted view that effete epithelial cells are simply exfoliated into the lumen. In order to explain this discrepancy, the present study examined luminal cell elements of the small intestine in the guinea pig, rat and mouse; the latter two have been favored species for studying the kinetics of intestinal cells.

Light and electron microscopic observations indicated that the luminal fluid of the guinea pig contained numerous cytoplasmic fragments covered with long microvilli and not containing a nucleus; these fragments corresponded with the apical cytoplasm of apoptotic epithelial cells. In the rat and mouse, in contrast, luminal cell elements were represented by round cell bodies possessing a nucleus and microvillous border; the nucleus displayed compaction and segregation of chromatin at the periphery, a microscopic figure characteristic of apoptosis.

As far as the rat and mouse are concerned, the present findings support the accepted view that epithelial cells undergoing apoptosis are exfoliated as total, nucleus-containing cells. In the guinea pig, in contrast, only an apical thin plate of effete cells is shed off, as our previous studies have suggested.

Epithelial cells in the gut originate in crypts, move toward the villus tips and eventually are extruded into the lumen as evidenced by autoradiographic studies using H-thymidine (LEBLOND, 1981). This process includes the natural death of the epithelial cells at the villus tips, which occurs so regularly that it must be classified in apoptosis in the sense of programmed cell death (WYLLIE et al., 1980; KERR and HARMON, 1991).

Our previous electron microscopic study (HAN et al., 1993) demonstrated a process of destruction and elimination of effete epithelial cells at the villus tip in the small intestine of the guinea pig. In this species it was noteworthy that the effete enterocytes were not simply exfoliated into the lumen, but were damaged by intraepithelial large granular lymphocytes (LGLs) to be thereafter phagocytosed by subepithelial macrophages; only a thin apical cytoplasm with a microvillous border remained intact to be pushed by adjacent epithelial cells and pinched off into the lumen (HAN et al., 1993; IWANAGA, unpublished data). Thus, the large, basal cell body is eroded away and the remaining apical cell cortex preserves the intestinal barrier until the space is filled by viable epithelial cells. This hitherto unknown process demonstrated in the guinea pig sharply contrasts with the generally accepted view on the fate of effete enterocytes (LEBLOND, 1981).

Our preliminary study suggested that the process of the disposal of effete enterocytes was not identical among species; the phenomenon observed in the guinea pig most likely occurred in the monkey and possibly in humans. However, the process found in the guinea pig could not be confirmed in the rat and mouse, two of the most favored animals for studying cell kinetics of gut epithelial cells. In order to determine more precisely the mechanism of the disposal of effete enterocytes and its species-differences, the present study

*This work was supported by a grant from the Research Foundation of Tachikawa General Hospital, Nagaoka, Niigata.
foci on the morphological and cytochemical analysis of luminal cell elements which were collected from the small intestine of the guinea pig, rat and mouse.

MATERIALS AND METHODS

Five male Hartley guinea pigs (body weight 250-300 g), five male Wistar rats (body weight 200-250 g) and five male ICR mice (25-30 g) were used in this study. After starvation for 24 h, the animals were anesthetized by inhalation of halothane and the abdominal cavity was opened. Empty portions of the ileum, 3-7 cm in length, were looped and injected with 1-2 ml of physiological saline. Five minutes later, the intraluminal fluid was collected and centrifuged at 3000 rpm for 5 min. The supernatant in the centrifuging tubes was discharged and the precipitate was fixed for either light or electron microscopy.

For light microscopic observation and histochemistry, the precipitate was fixed for 30 min with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After fixation, it was washed with saline and centrifuged again at 3000 rpm for 10 min. The emulsion, rich in cell elements, was dropped on poly-L-lysine-coated glass slides and dried in the air.

Some preparations were stained with hematoxylin and eosin, whereas others were histochemically treated for detection of F-actin, a marker substance for microvilli of epithelial cells. In the latter staining, the preparations were incubated for 30-60 min with rhodamine-labeled phalloidin diluted in 1:20, which is specifically bound to F-actin (WULF et al., 1979). After washing, the preparations were embedded with glycerin and examined under a Leitz Ortholux equipped with a fluorescence vertical illuminator (Ploemopak 2.2). The specimens, after being photographed, were restained with hematoxylin in order to observe identical locations under a conventional light microscope; some preparations were directly examined with a phase-contrast microscope.

For electron microscopy, centrifuged precipitates were fixed for 2 h with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4 and cut into small pieces. After washing, they were postfixed in 1% OsO₄ for 1.5 h. Specimens were dehydrated through a graded series of ethanol and embedded in Araldite via propylene oxide. Ultrathin sections were stained with uranyl acetate and lead citrate, to be examined under a Hitachi H-7000 transmission electron microscope.

RESULTS

Light microscopic observation of luminal cell elements

Guinea pig: Luminal cell elements obtained from the ileum contained only a few cells with a nucleus, most of them being neutrophils and lymphocytes. More numerous than these blood-born contaminants were cell fragments without a nucleus (Fig. 1a). The cytoplasmic fragments were smaller in size (4.58 ± 0.09 μm in large axis, n=50) than erythrocytes, and varied in shape; most of them were globular or hemispheric bodies, or curved plates. In the globular bodies, their whole surface was covered with radially arranged microvilli. The hemispheric bodies and curved plates were equipped with such elongated microvilli only on their convex surface. The microvilli were markedly longer than those of the enterocytes within the epithelium as seen in tissue sections.

Rat and mouse: Numerous rounded cells containing a nucleus were found in the intraluminal cell elements of the rat and mouse (Fig. 2). The cells possessed a rich cytoplasm and were 10.07±0.20, μm (n=50) and 9.44 ± 1.33, μm (n=50) in size in the rat and mouse, respectively. Most of the cells were single cells, though occasional ones were grouped in two or three cells. Their cell surface was covered with regularly arranged microvilli; the extent of the microvillous portion and the detailed morphology of the microvilli were unclear in the preparations (see below). The nucleus displayed compactly accumulated chromatin distributed either in a crescent at the periphery or in small, round bodies (Fig. 2).

Rhodamine-phalloidin staining of luminal cell elements

Guinea pig: When the intraluminal cell elements were incubated with rhodamine-phalloidin, bright fluorescence indicating the existence of F-actin was specifically recognized in small, round or irregular-shaped bodies (Fig. 1b). Observation at higher magnification demonstrated that the fluorescence corresponded to a tuft of long microvilli growing on those bodies. When the fluorescent bodies were examined with a conventional light microscope or phase-contrast microscope, they were confirmed to be cytoplasmic fragments which were covered with microvilli and lacked a nucleus (Fig. 1).

Rat and mouse: Large, round cells intensely fluoresced in their microvillous portion on the cells floating in the intestinal fluid (Fig. 3). The fluorescent border covered the cell surface, ranging from one
Fig. 1. Luminal cell elements obtained from the ileum of a guinea pig. a is counterstained with hematoxylin.
Four cellular fragments (arrows) are found to be covered with long microvilli and lack a nucleus. A nucleus-containing cell indicated by an arrowhead is a lymphocyte. These four cellular fragments intensely fluoresce to rhodamine-phalloidin (b). a, b: ×1,400
Fig. 2. Luminal cell elements of the rat ileum. Hematoxylin-eosin stain. All cells possess a nucleus in which chromatin is compactly accumulated on the nuclear envelope. Some of the cells display a striated border on their surface (arrows). ×1,400

Fig. 3. Luminal cell elements of the rat ileum stained with rhodamine-phalloidin. Fluorescent microvillous portion covers some or all of the cell surface. ×1,400
third to almost the entire length of the circumference. The striated border tended to be smaller in thickness and weaker in reaction when it covered a broad cell surface surpassing half of the whole circumference (Fig. 3). Conventional and phase-contrast microscopy allowed demonstration of the typical apoptotic nucleus mentioned above in the cells in question.

**Electron microscopic observation of luminal cell elements**

_Guinea pig_: Numerous globular or irregularly shaped bodies with long microvilli were found in the precipitates of the intraluminal fluid (Fig. 4). The microvilli were markedly elongated (1.4–2.0 μm against the normal length of 0.9–1.0 μm) and covered the convex surface of the cytoplasmic bodies; in the globular bodies, they circled the entire length of the surface (Fig. 4). The microvilli tended to taper towards their tip; occasional ones with a thick basal portion showed branching. The microvilli contained a bundle of actin filaments which penetrated deeply into the terminal web region of the cytoplasm. The intracellular extensions of the actin bundles, called rootlets, were at least twice as long as those in intact enterocytes in the epithelial lining. The cytoplasm, except for the terminal web region, was rich in clear vesicles of various sizes and contained a small number of mitochondria. It frequently possessed one or two round or irregular-shaped cytoplasmic inclusions, possibly lipid droplets, measuring more than 1 μm in diameter. They lacked a limiting membrane, and were homogenous in structure and moderately electron-dense (Fig. 4).

_Rat and mouse_: In the rat, cells possessing rich cytoplasm and a nucleus were predominant in the luminal fluid examined by electron microscopy. The intraluminal cells could be identified as enterocytes from their closely arranged microvilli and ultrastructural characteristics of cell organelles (Fig. 5). The microvilli were irregular in arrangement and length as compared with those of intact enterocytes within the epithelium. In addition to mitochondria and endo-

---

**Fig. 4.** Electron micrograph showing cytoplasmic fragments found in the intraluminal fluid of the guinea pig ileum. The cytoplasmic fragments possess a microvillous portion composed of long microvilli. Mitochondria and vesicular elements are recognizable in the fragments. E erythrocytes, L lipid droplet. ×6,400
Figs. 5 and 6. Legends on the opposite page.
plasmic reticulum, the cytoplasm was rich in vesicles which either were electron-lucent or possessed an electron-dense core. Lipid droplets of various sizes and lysosomes were also scattered throughout the cytoplasm. Chromatin was condensed to form dense masses at the periphery of the nucleus; in many cells, the nuclear outline was convoluted and protruded into the cytoplasm due to the aggregation of chromatin (Fig. 5). Occasionally, chromatin displayed characteristic figures of one or two crescent masses along the nuclear membrane.

In the mouse, ultrastructures of the luminal cell elements were essentially identical to those in the rat, except that they were intermingled with nucleus-free cytoplasmic fragments (Fig. 6). The latter comprised round membrane-bounded cytoplasm, some of them being equipped with microvilli. The cell fragments resembled the nucleus-containing cell elements in composition of the organelles.

**DISCUSSION**

In the present study, luminal cell elements of the small intestine were investigated by light and electron microscopy in the guinea pig, rat and mouse. The cellular elements were easily collected from the lumen in all species, and their morphological analysis was shown to be useful for studying the fate of effete epithelial cells. Conventional observation at light and electron microscopic levels allowed plentiful information on this. Moreover, histochemistry with rhodamine-labeled phalloidin, which specifically binds to F-actin (WULF et al., 1979), proved helpful in identifying enterocytes, due to a clear reaction of the microvillous border unique to these. The results showed that the figure of exfoliated cell elements differed between the guinea pig and the rat/mouse, suggesting a different mechanism for the exclusion of apoptotic epithelial cells.

In apoptosis, cell bodies are usually fragmented and form membrane-bounded small corpuscles, called apoptotic bodies, which are then either extruded into an adjacent lumen or phagocytosed by resident tissue cells (WYLLIE et al., 1980). In fact, the effete enterocytes in the guinea pig small intestine were fragmented before the phagocytosis by macrophages and formed membrane-bounded cytoplasmic bodies (HAN et al., 1993). That a typical process of apoptosis was taking place in aged enterocytes in the rat and mouse was supported by the compaction and segregation of chromatin demonstrated in the luminal cell elements, since this change in nuclei is known to characterize apoptosis (WYLLIE et al., 1980; KERR and HARMON, 1991). The coexistence of membrane-bounded cytoplasmic fragments with apoptotic cells in the mouse suggests the fragmentation of the cytoplasm possibly occurring in the process of apoptosis in a certain portion of aged enterocytes. It remains to be determined whether cell fragmentation might occur within the epithelium or in the lumen.

The difference in the fashion of the disposal of apoptotic enterocytes between the guinea pig and the rat/mouse should be linked to the occurrence of LGLs in the epithelium and to the aggregation of macrophages in the subepithelial region at the villus tips. In the guinea pig, macrophages gathering beneath the epithelium actively phagocytose the enterocyte bodies, which apparently have been pretreated by the LGLs. Macrophages in the rat, though numerous, are weak in phagocytic activity, and those in the mouse are very few in number (HAN, 1993). In the monkey.

---

Figs. 5 and 6. Luminal cell elements from the ileum of rat (Fig. 5) and mouse (Fig. 6). The cells in the rat contain a nucleus (N) in which chromatin is accumulated at the periphery into segregated masses. In the mouse, small, round cytoplasmic fragments are intermingled with the nucleus-possessing segregated elements. Note that chromatin masses elevate the outline of the nucleus (N) into the cytoplasm. LD lipid droplets Ly lysosomes M microvilli

Fig. 5: ×7,100, Fig. 6: ×5,200
and horse, large aggregations of intensely phagocytotic macrophages in the lamina propria as well as the occurrence of LGLs in the epithelium were also recognized in the small intestine, suggesting the existence, in these species, of the same mechanism for the disposal of effete epithelial cells as in the guinea pig.

The functional significance of the species-difference as to the fashion of the disposal of effete enterocytes remains unknown. In the guinea pig, the skin-like apical cytoplasm remaining after a large loss of cell bodies is interpreted as a device for maintenance of the epithelial barrier until the loss is filled up by neighboring epithelial cells (IWANAGA, unpublished data). In the rat and mouse, on the other hand, enterocytes are exfoliated into the lumen as an entire cell containing a nucleus. Therefore, it seems possible for the epithelial barrier to be destroyed even for a short time (TRAVIS and MENZIES, 1992). RITTER (1957) reported that, under a light microscope, gaps are left after effete enterocytes have been exfoliated. Some researchers have maintained that large molecular substances can permeate through such epithelial gaps (CLARKSON, 1967). Other researchers have disputed this view, insisting that normal exfoliation of effete enterocytes does not cause disruption in the barrier. MADARA (1990) suggested that dynamic alternations in the intercellular junctional devices at the cell extrusion sites might prevent the loss of the barrier function. He postulated that newly formed junctional elements might function as a zipper in closing the space for extruding cells, thus preventing epithelial discontinuities from occurring.

REFERENCES


