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Macrophages Possibly Involved in the Disposal of Apoptotic Epithelial Cells in the Monkey Small and Large Intestine

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Summary. **The tips of villi in the monkey small intestine are demonstrated to contain a large, dense aggregation of macrophages in the lamina propria. They are intensely positive for acid phosphatase and, under the electron microscope, reveal numerous phagosomes whose contents resemble various portions of effete epithelial cells. The macrophages extend pseudopods to phagocytose apoptotic epithelial cells including goblet cells. Lymphocytes showing morphological features of LGLs (large granular lymphocytes) are dispersed in the epithelium of the villus tip.**

In the colon smaller macrophages with more inconspicuous phagosomes are seen loosely gathered beneath the superficial epithelium. Large macrophages, on the other hand, are aggregated around the cryptal base as well as between the lamina muscularis mucosae and an underlying lymphatic. The rectum, in turn, shows rather dense aggregations of macrophages beneath the superficial epithelium, forming intercryptal cell cords.

This study suggests that in the monkey, as previously demonstrated in the guinea pig, effete epithelial cells are disposed of by macrophages, likely assisted by the cytotoxic activity of LGLs. The role of macrophages in the elimination of apoptotic epithelial cells is evident in the small intestine but less so in the large intestine.

INTRODUCTION

It has been widely known that the lamina propria of mammalian small and large intestine contains numerous macrophages which increase in pathological, especially inflammatory, or experimentally stimulated conditions.[1-5] Möllendorff[1] and Maximow[6] noted that in normal guinea pigs, numerous macrophages are aggregated at the tip of villi of the small intestine. This peculiar phenomenon was forgotten for a long time to be reconfirmed by modern electron microscopists, Sawicki and associates,[7] again in this particular animal, the guinea pig. These authors simply mentioned that macrophage aggregation was much less marked in the rat, mouse and humans; they examined no other species.

Concerning the large intestine, the human colon and rectum are known to contain numerous macrophages in the subepithelial portion of the lamina propria.[8,9] Information in animals is rather sparse. In the guinea pig, the above-mentioned researchers, Sawicki and associates,[7] extended their study to the large intestine and recorded that macrophages were densely gathered in the subepithelial layer of the lamina propria. In the rat and mouse, they found less marked macrophage aggregation in corresponding sites.

As reviewed above, previous knowledge concerning the subepithelial macrophage aggregation in the gut has been very poor, essentially because of the limited number of species examined. As far as the functional significance of this macrophage aggregation is concerned, the views of previous authors diverge. Many researchers seem to interpret it as an inflammatory or immunoreactive change,[3,4,10] while some others regard it a normal phenomenon. Möllendorff[6] and Maximow[1] thus suggested that the subepithelial macrophages might take up certain substances from the intestinal lumen. Sawicki et al.[7] proposed that they might be involved "in the phagocytosis of some migrating cells of the intestinal mucosa, most probably of the sheathfibroblasts and/or intraepithelial lymphocytes". The "sheathfibroblasts" are flat cells extending closely beneath the basement membrane and are known to "migrate" or shift in the same direction with the epithelial cells.[11] From a similar viewpoint, Kobayashi et al.[12] suggested that the macrophages in the villus tip might be involved in the disposition of nerves, which constant-

ly grow towards the villus tip as well.

We recently confirmed the normal, constant occurrence of macrophage aggregation in the villus tip of the guinea pig small intestine under the light and electron microscopes[13]. The fine structural observations of phagosomes in the macrophages strongly suggest that what are disposed of by the macrophages constitute, if not exclusively, the epithelial cells of the intestine. This idea was supported by our experiment using bromodeoxyuridine (BrdU). The epithelial cell nuclei labeled with BrdU were traced to shift towards the villus tip and later to be incorporated into the subepithelial macrophages.[13]

We then demonstrated that macrophages were also aggregated, as Sawicki et al.[7] showed, in the lamina propria of the large intestine of the guinea pig. Here they formed distinct cords between individual crypts. Electron microscope observations again indicated that the epithelial cells were phagocytosed by the macrophages.[14]

While textbooks maintain that effete epithelial cells are exfoliated into the lumen at the tip of the villi in the small intestine and in the intercryptal zone in the large intestine,[15] the above findings of our research group support a novel view that aged epithelial cells are taken up, from the interstitial side, by macrophages. Our previous studies[13] in the guinea pig further demonstrated that large granular lymphocytes (LGLs), most probably endowed with natural killer (NK) cytotoxicity, occur numerously in the epithelium in question to destroy and erode the effete cells; the latter thus fragmented are then phagocytosed by the macrophages.

In order to examine whether the phenomena observed in the guinea pig might occur in other species, we are extending light and electron microscopic surveys to other mammalian species. The present paper demonstrates our findings in the small and large intestine of the monkey. A part of this study has been briefly introduced elsewhere.[14]

MATERIALS AND METHODS

Seven Jananese monkeys, *Macaca fuscata*, of both sexes, weighing 2.5–10 kg, were used in this study. The animals were anesthetized with pentobarbiturate and perfused via the aorta with a physiological saline followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Various regions from the duodenum to the rectum were obtained and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for 6 h. The tissues were dipped in 30% sucrose solution overnight at 4°C and rapidly frozen in liquid nitrogen. Frozen sections, about 20 μm in thickness, were prepared in a cryostat (Coldtome CM-41, Sakura, Japan) and stained for the detection of an acid phosphatase activity, according to Burnstone.[16]

For electron microscopy, perfusion-fixed materials were dissected out into small pieces and immersed in 2.5% glutaraldehyde solution for an additional 2 h. The tissue blocks were then post-fixed in 1% OsO_4 dissolved in phosphate buffer for 1.5 h, dehydrated through a series of graded ethanol and embedded in Araldite via propylene oxide. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a Hitachi H-7000 transmission electron microscope.

OBSERVATION

1. Light microscopy

Macrophages, especially their aggregations could be identified in hematoxylin and eosin-stained sections, due to their rich eosinophilic cytoplasm. Acid phosphatase reaction stained the cells intensely and selectively; this was useful for a survey of the distribution of macrophages in different portions of the intestine (Figs. 1–4).

Small intestine

Numerous macrophages were found in the lamina propria of the villi. They increased in number and size upwards and, at the villus tip, large rounded macrophages were densely gathered in a large cell mass (Figs. 1, 2). Every villus, from the duodenum down to the ileum, contained a macrophage aggregation on essentially the same scale. From 20 to 30 macrophages were generally counted in one villus tip.

The macrophages at the villus tip were large, rounded forms (30–40 μm in diameter) containing many granular bodies which reacted positively for acid phosphatase. Besides those in the lamina propria, a considerable number of macrophages could be found within the epithelium (Fig. 2).

In the lower half of the villi macrophages were seen around the central lacteal. These were, however, much fewer, smaller, more elongated and weaker in acid phosphatase reaction, as compared with those near the tip (Fig. 1). They did not show any sign of invading the epithelium.

A few clear cells, regarded as lymphocytes, were found in the villous epithelium. They were more

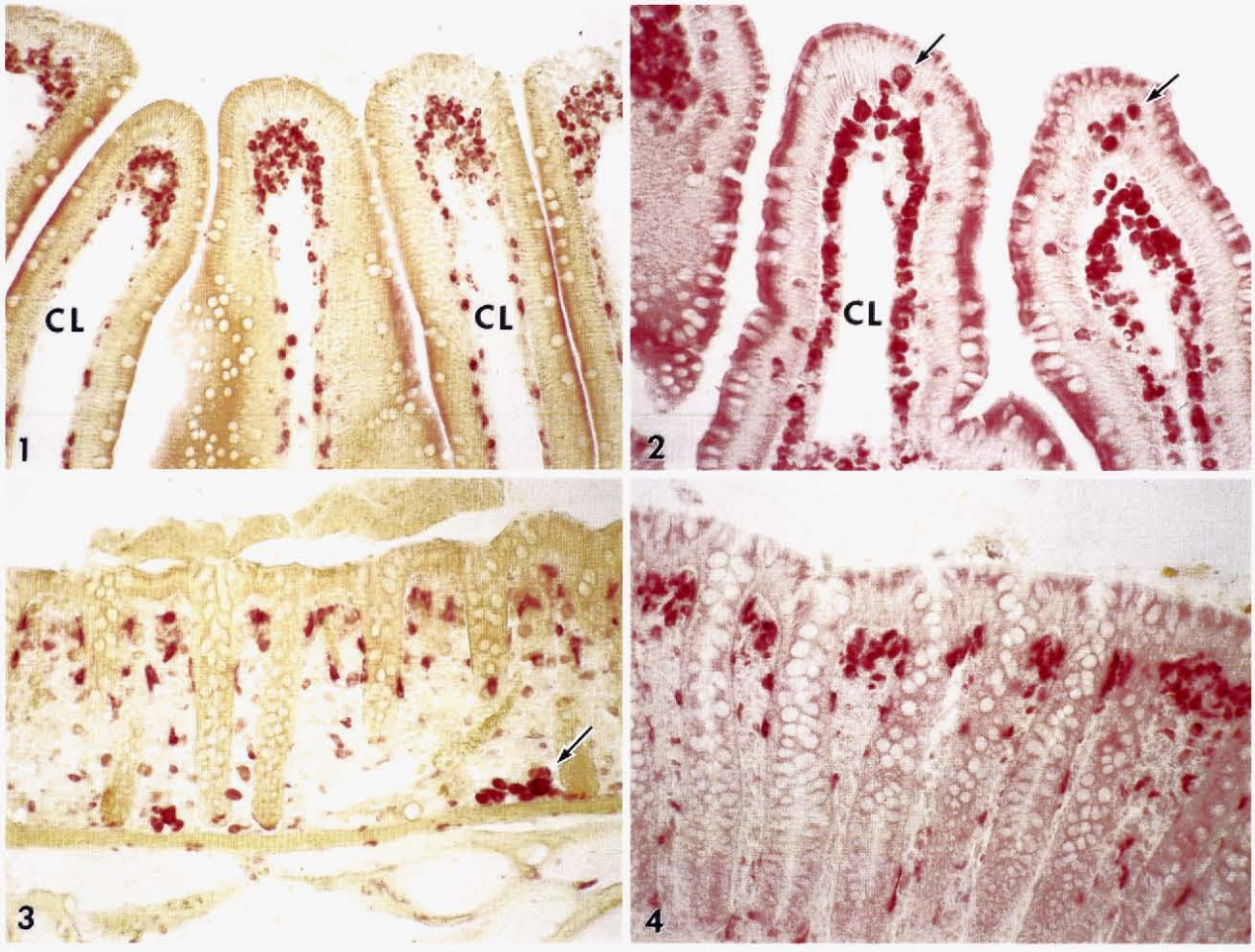


Fig. 1. Monkey duodenum. A cryostat section stained for acid phosphatase. Aggregations of acid phosphatase-positive macrophages are found in the tip of every villi. Macrophages with less intense activity for acid phosphatase are scattered along the central lacteals (CL).

Fig. 2. Large and round macrophages line the subepithelial region of the monkey jejunum. Some macrophages (arrows) invade the epithelium of villus tips. CL: central lacteal

Fig. 3. In the colonic mucosa of a monkey, macrophages with less intense activity for acid phosphatase are dispersed in the lamina propria. Aggregations of intensely positive macrophages (arrow) are recognizable around the bottoms of crypts.

Fig. 4. Monkey rectum. Small but conspicuous aggregations of macrophages are seen under the superficial epithelium between the crypts.

numerous in the upper portion, especially at the tip of the villi.

Colon

Many macrophages were found closely beneath the superficial and cryptal epithelium. They were especially numerous under the superficial epithelium. Even here, however, the macrophages were gathered only loosely and could not be called a cell aggregation; they were rather attenuated and stellate in profile (Fig. 3). Intraepithelial macrophages were difficult to recognize.

On the other hand, aggregations of large round macrophages containing large, strongly acid phosphatase-positive bodies were recognized in the lamina propria at the bottom of the crypts (Fig. 3) and, more conspicuously, directly beneath the lamina muscularis mucosae (not shown in the figure). These aggregations did not occur continuously along the muscularis but rather formed patches here and there.

Such a patch of macrophages was usually located between the muscularis and an underlying lymphatic vessel (corresponding electron micrograph: Fig. 10).

Lymphocytes were rather rarely recognized in the epithelium of the colon.

Rectum

Macrophages of a moderate size and oval shape were concentrated fairly densely under the superficial epithelium of the rectum (Fig. 4). They were intensely stained in acid phosphatase reaction. Smaller macrophages with a weaker reactivity were dispersed along the cryptal epithelium, gradually becoming fewer towards the cryptal bottom. In the rectum, no such a macrophage accumulation close to the muscularis or to submucosal lymphatics as was seen in the colon could be found.

The occurrence of a few lymphocytes in the superficial epithelium was suggested, but they were generally difficult to be identified due to the occurrence of numerous goblet cells.

2. Electron microscopy

Macrophages disposed in different portions of the gut and different sites in the mucous membrane showed marked differences in cytoplasmic structures, among others in the amount and contents of phagosomes, as revealed under the electron microscope. In the small intestine, however, the findings on macrophages, as well as lymphocytes, were rather uniform from the duodenum to the ileum, and so will be treated collectively.

Small intestine

Macrophages in the lamina propria of villus tips in the small intestine contained, besides numerous lysosomes, larger and smaller phagosomes showing a variety of contours and contents (Figs. 5, 6). Larger ones surpassed the size of the nucleus of the cell. The

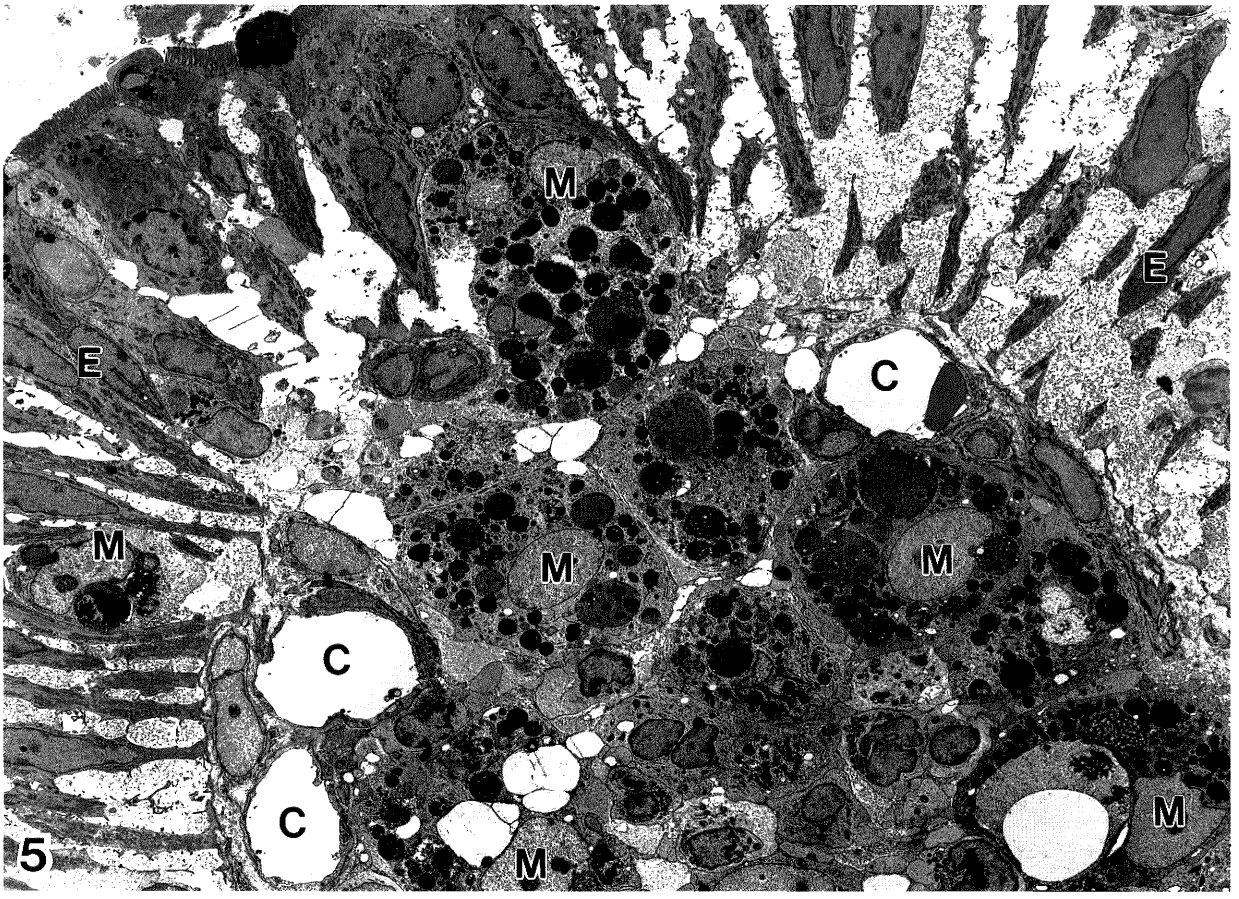


Fig. 5. Electron micrograph showing a villus tip of the ileum. Numerous macrophages (M), rich in phagosomes of various sizes, are gathered in the subepithelial region. Two macrophages invade the epithelial lining (E). C: capillary ×2,000

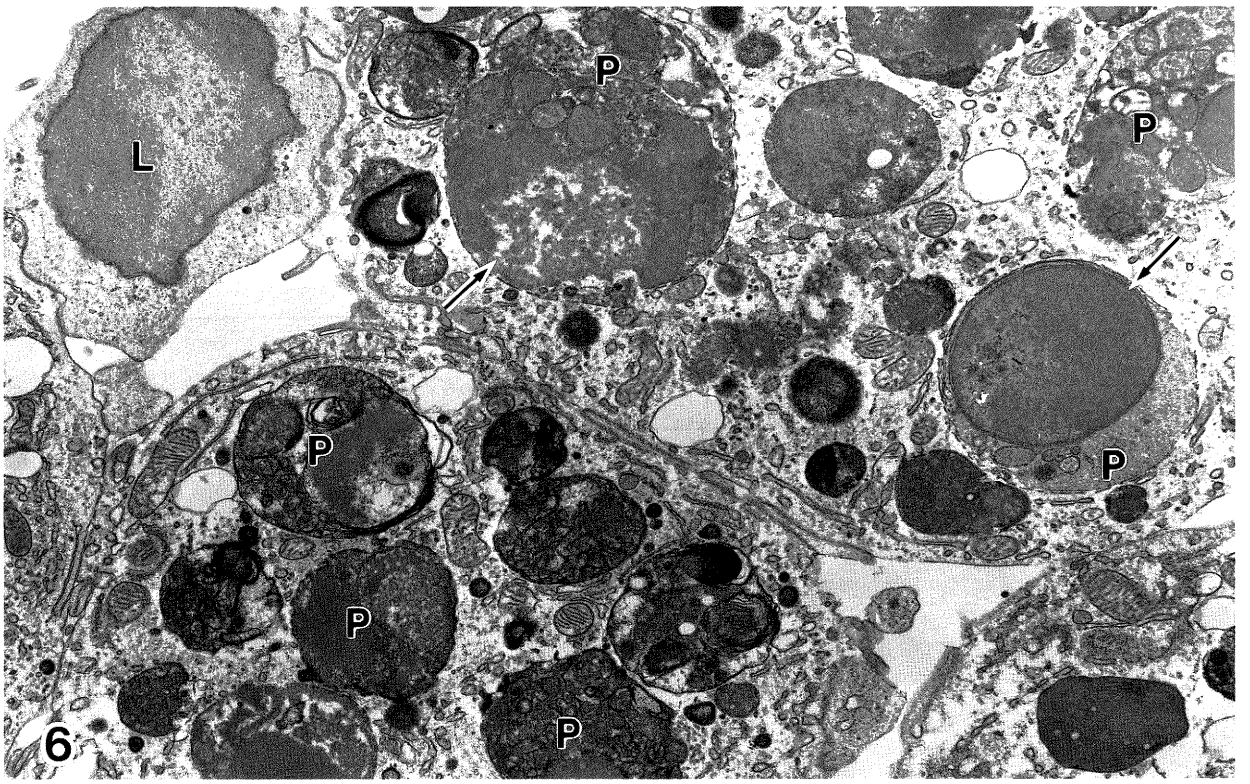


Fig. 6. Closer view of macrophages seen in the lamina propria of the ileum of a monkey. The cytoplasm is filled with large phagosomes (P), in which ingested cellular elements such as mitochondria and nuclei (arrows) are recognizable. L: lymphocyte ×10,000

contents of phagosomes comprised cellular elements which were regarded to have been phagocytosed by the cell. They included mitochondria, nuclei and some other elements. In most phagosomes, however, we could not identify such elements as they were represented by a mass of condensed and presumably digested materials (Fig. 6). The electron density of phagosomes varied conspicuously, ranging from very dark homogeneous bodies to clear vacuolated ones.

Some macrophages extended a pseudopodium into the epithelium (Fig. 7), while a few others were located completely within the epithelium.

Lymphocytes, though not very frequent, were found in the epithelium at the villus tip. They were either rounded in contour or extended one or more pseudopodia among the epithelial cells. Most of the lymphocytes revealed a few round granules of characteristically high electron density measuring 300–600 nm in diameter, thus constituting cells deserving to be called large granular lymphocytes (LGLs) (Fig. 8).

The epithelial cells, including goblet cells and a few basal-granulated cells, were more and more widely separated from each other as they approached the villus tip (Figs. 7, 8). In their apical portion, however, the epithelial cells were tightly juxtaposed and combined with junctional complexes.

Colon

The macrophages gathering beneath the superficial epithelium of the colon contained numerous phagosomes as well, but these were smaller in size than in the macrophages in the small intestinal villi. Moreover, most of the phagosomes comprised densely packed materials which occasionally revealed altered images of mitochondria and some other organelles, and we could obtain little evidence supporting that they might derive from effete epithelial cells phagocytosed by the macrophages (Fig. 9). Neither could we encounter electron microscopic images suggesting an invasion of macrophages, either with a pseudopodium or as a whole cell, into the epithelium.

The macrophages beneath the lamina propria mucosae were larger cells containing larger and more numerous phagosomes than the subepithelial ones mentioned above (Fig. 10). The phagosomes

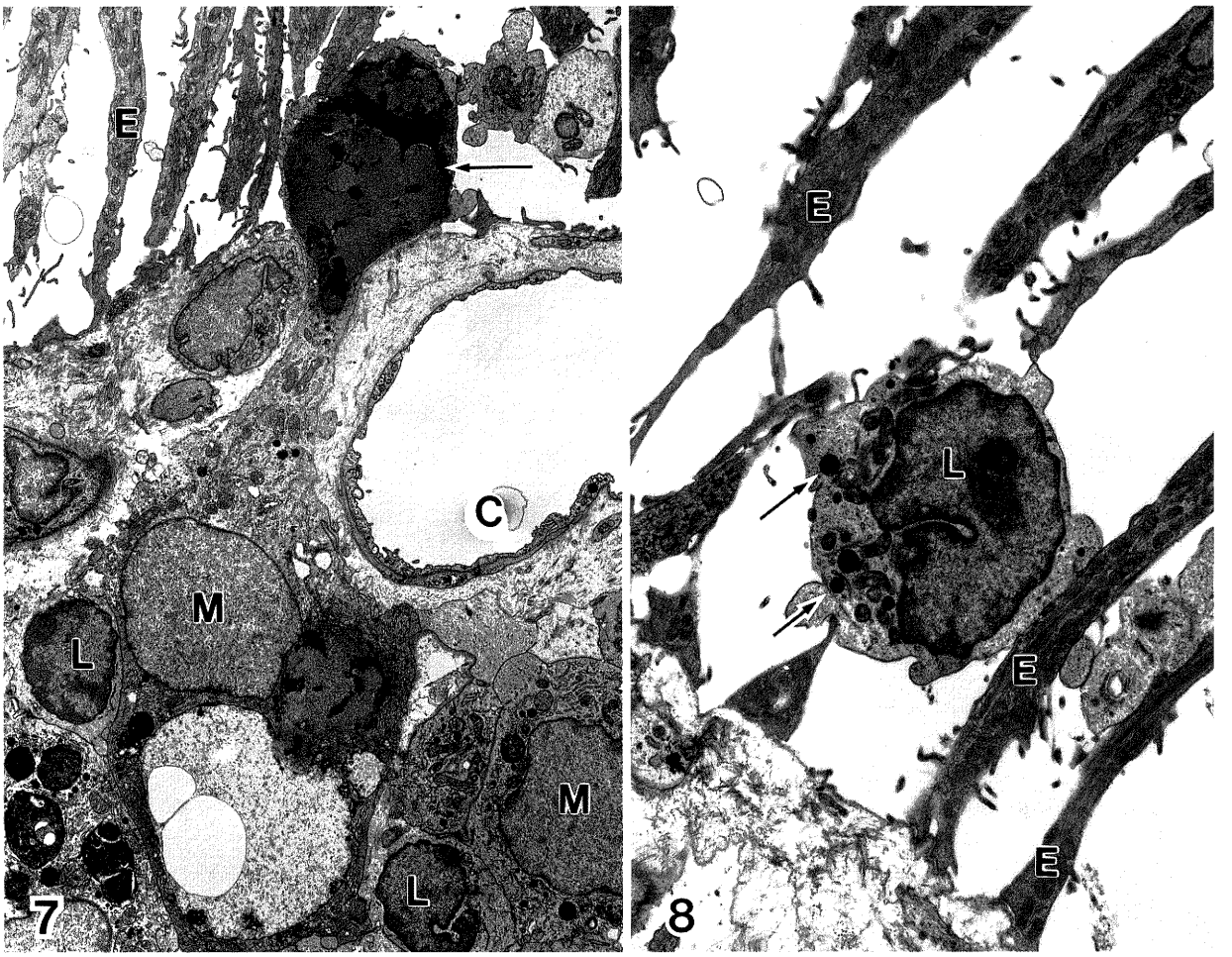


Fig. 7. The villus tip of the ileum. A macrophage (M) in the lamina propria extends a pseudopod into the epithelial lining (E). Cellular elements, possibly derived from a goblet cell, are seen in the pseudopod (arrow). C: capillary, L: lymphocytes ×3,500

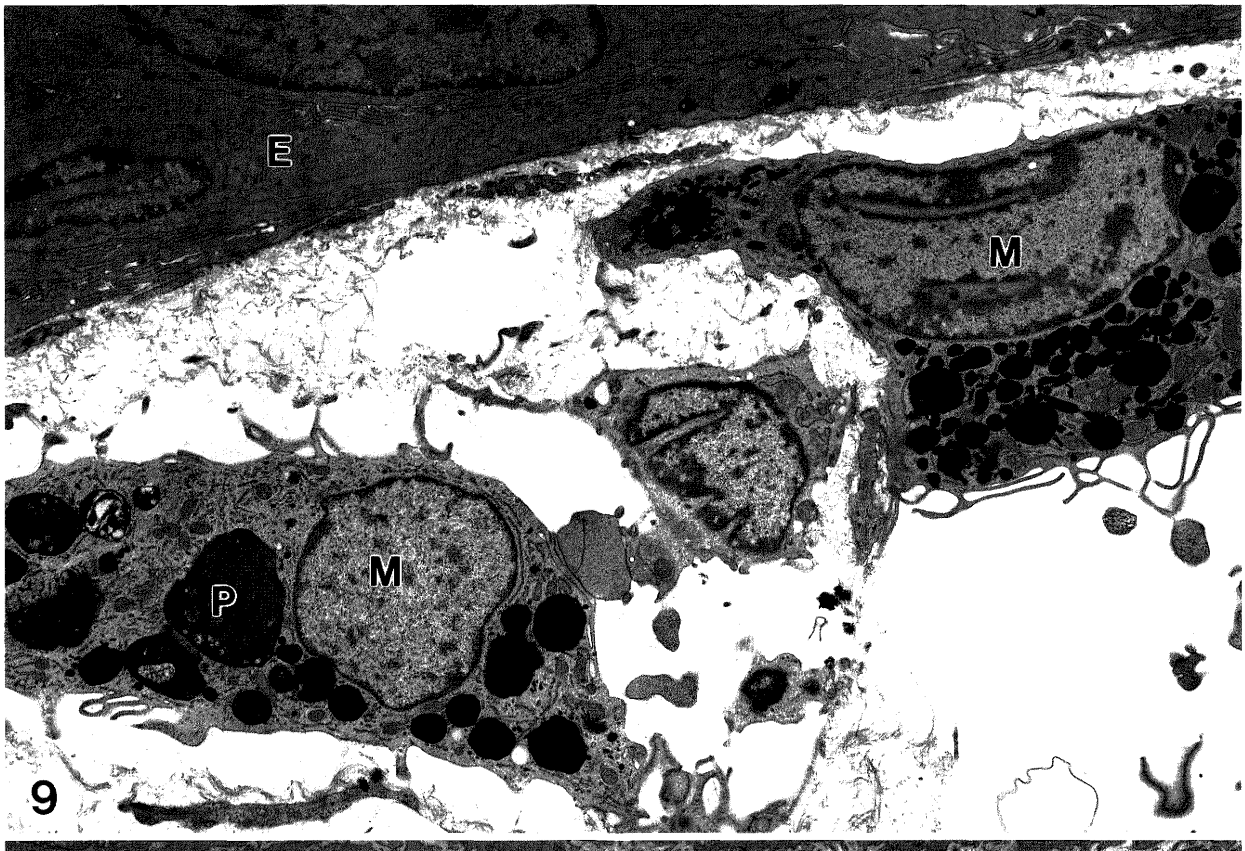
Fig. 8. A lymphocyte (L) in the epithelium of the ileum contains several electron-dense granules (arrows) in the cytoplasm, characteristic for an LGL. E: the basal cytoplasm of enterocytes. ×8,100

Fig. 9. Macrophages in the colonic mucosa of a monkey. Two macrophages (M) are seen in the lamina propria close to the superficial epithelium (E). They also contain many phagosomes (P) or residual bodies, most of which are homologous and electron-dense. ×6,700

Fig. 10. An aggregation of macrophages (M) in the tela submucosa of the colon. It is located between the lamina muscularis mucosae (LM) and a lymphatic vessel (LV). The cytoplasm of macrophages is rich in phagosomes which are smaller in size than those of lamina propria macrophages. L: lymphocytes. ×2,700

were highly variable in size, shape and electron density. Many of them revealed contents reminiscent of mitochondria and other cellular elements, but their origin remained unclear.

The macrophages were aggregated densely, in some parts directly contacting each other. Upon facing a lymphatic lumen, the cells were directly encapsulated by an attenuated sheet of a fibroblast and this, in its turn, paralleled the endothelium over a thin interstitium (Fig. 10). We could find no indication of a macrophage migrating into the lymphatic or projecting a process into it. The aspect of the macrophage aggregation facing the lamina muscularis was often intervened by irregular profiles of lymphatic spaces incompletely lined by a thin endothelium-like cell sheet (Fig. 10).


E
M
P
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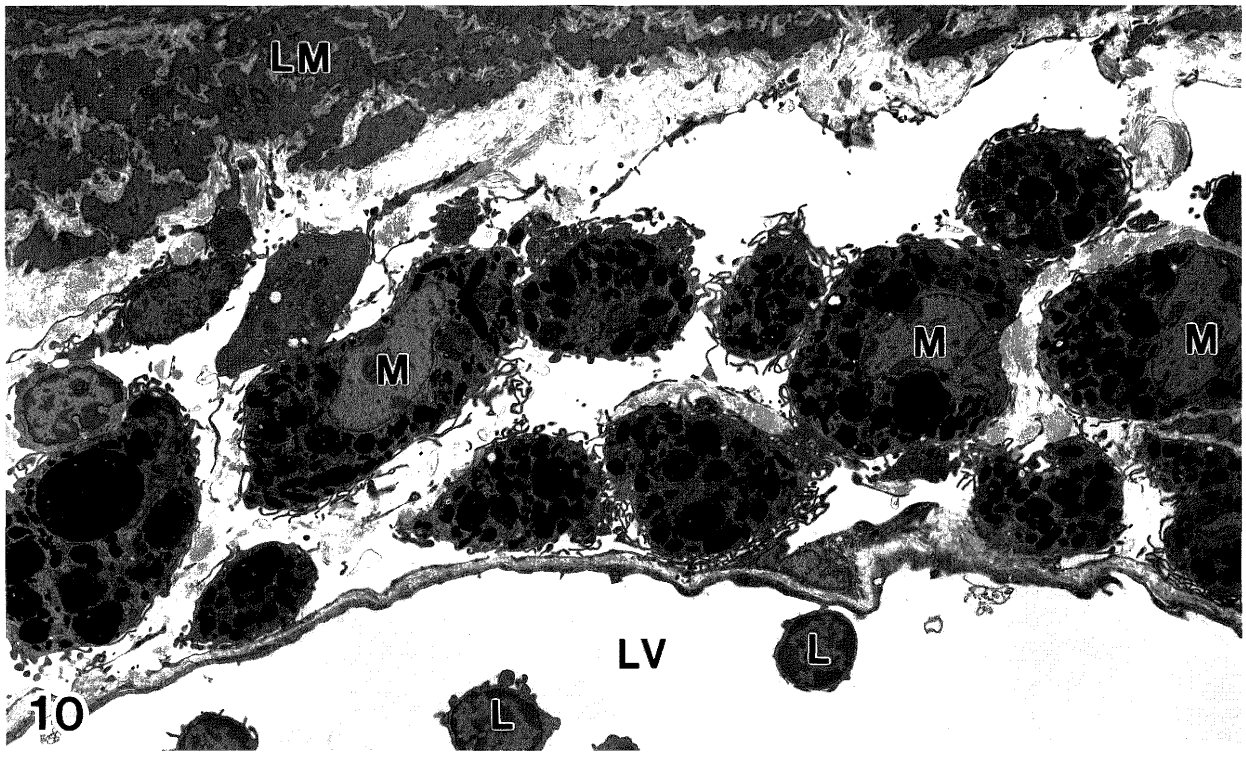
9


LM
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LV
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10

The rectum was not observed by electron microscopy in the present study.

DISCUSSION

Small intestine

The present study demonstrates both by light and electron microscopy that, in the monkey small intestine, macrophages are densely aggregated in the tip of villi. We have recorded a brief account of this finding elsewhere.[14] Macrophage aggregations, closely resembling and even more conspicuous than those in the present finding in the monkey, have been reported by us in the same villus tip in the guinea pig small intestine.[13]

As in the guinea pig,[13] the macrophages in question in the monkey reveal under the electron microscope numerous phagosomes which contain internalized cell fragments. Although these phagosomes in the monkey generally are smaller in size and their contents are less clearly identifiable than those in the guinea pig, it is most probable that they represent portions of villous epithelial cells which have been phagocytosed and are being digested by the macrophage; the possibility is not excluded that they also contain portions of other cells like fibroblasts[7] and nervous elements[12] becoming apoptotic in the terminal region of villi.

Lymphocytes, though not so numerous as seen in the guinea pig,[13] appear in the monkey small intestine, being dispersed in the villous epithelium, especially at the tip. Their being LGLs possessing natural killer cytotoxicity is suggested on the basis of their fine structure, exemplified by their dense granules.[17] This finding and interpretation lie in accord with those reached by us in the guinea pig.[13] In the guinea pig, electron microscopic images could be collected suggesting that the LGLs vigorously eroded and split the epithelial cells at the villus tip; the subepithelial macrophages seemed to phagocytose the now apoptotic and fragmented epithelial cells more easily than otherwise.[13] In the case of the monkey examined in the present study, the lymphocytes' lytic and splitting effect upon the epithelial cells could not be definitively demonstrated. Only it was noticed that LGLs tended to be surrounded by more space-rich epithelium than elsewhere.

Colon

In our previous study in the guinea pig colon, large macrophages with numerous phagosomes were seen densely gathered beneath the superficial epithelium forming a distinct zone between individual crypts.[14] We interpreted this to mean that the macrophages were involved in the phagocytosis of effete epithelial cells which shifted to the intercryptal zone.[14] At the corresponding site in the colon of the monkey, on the other hand, we could demonstrate only macrophages of a smaller size and shrunken shape containing rather inconspicuous phagosomes. It seems therefore difficult to suggest the same role for these cells as in the guinea pig. Moreover, we could find only a few lymphocytes in the intercryptal epithelium.

In contrast, the present study demonstrated large, phagosome-rich macrophages aggregated between the cryptal base and an underlying lymphatic. In spite of their striking appearance both under the light and electron microscopes, no previous reports seem to have dealt with them. We could not suggest a possible role for these subcryptal macrophages, and failed to detect their migration into the lymphatic vessel.

Rectum

In the guinea pig, we found less marked and less typical macrophage aggregations in the rectum than in the colon.[14] In contrast, the present study in the monkey indicates that subepithelial macrophages in the rectum are more conspicuous, both in their size and in the size of their aggregations, than those in the colon. Although electron microscope observations in the rectum are missing in this study, it seems reasonable to suggest that effete epithelial cells might be disposed of by the phagocytotic activity of the subepithelial macrophages in the monkey rectum.

Remarks and conclusion

On the basis of the present electron microscope findings of the monkey tissue, and taking into account our previous results of electron microscopic analysis and a BrdU experiment in the guinea pig, we suggest that in the monkey, as we did for the guinea pig,[13] effete epithelial cells at the villus tip are phagocytosed by the subepithelially gathered macrophages. It is most likely that the aged epithelial cells are recognized and pretreated by the intraepithelial LGLs, though the monkey did not reveal such clear images indicating destructive activities of LGLs to the villous epithelium as the guinea pig did.[13]

The above idea is contrasted with the accepted view that effete epithelial cells of the gut are exfoliated as a whole. In the guinea pig, we have recently recognized that a small apical portion of the epithelial cells is left intact, while the cell body is destroyed

and replaced by an empty cavity. This "skin" carrying microvilli covers the defect and, while the latter is depressed and filled with surrounding cells, becomes gathered up into a small dome, which then is pinched and shed off. The intestinal barrier is believed to be preserved by this exquisite mechanism. In the monkey, it is the theme of a future study to investigate as to whether similar or different mechanisms might take place for the prevention of a rupture in the intestinal barrier during epithelial cell apoptosis.

Although our investigation in the monkey is not so detailed as in the guinea pig, mainly due to the availability of animals, the present data suggest the possibility that our new idea concerning the elimination of apoptotic epithelial cells by macrophages may hold true in another primate, the human being. It has been noted by us that aggregations of phagosome-rich macrophages in the villus tip are not recognizable in the rat, mouse and hamster, and it is likely that these laboratory animals have a different mechanism of epithelial cell disposal.

As to the large intestine of the monkey, the present observations seem insufficient to form a conclusion concerning the role of subepithelial macrophages and the mechanism of epithelial cell disposal. In the colon it seems unlikely for small-sized macrophages with inconspicuous phagosomes to take up epithelial cells. The findings in the rectum seem to support the possibility that effete epithelial cells are disposed of by the subepithelial macrophages, but further detailed studies including morphological analysis of their phagosomes and observation of the activities of lymphocytes are needed to draw a definite conclusion.

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