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ORIGINAL ARTICLES

ORIGINAL ARTICLE

THE ISSUE OF HISTOLOGICAL IDENTIFICATION OF M-CELLS IN THE PEYER'S PATCHES OF ALBINO RAT SMALL INTESTINE

DOI: 10.36740/WLek202205214

Volodymyr Hryn, Yurij Kostylenko, Natalia Svintsytska, Valentyna Bilash, Volodymyr Lytovka
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ABSTRACT

The aim: Based on the above cytological signs of M-cells, we set the goal of more detailed clarification of some of their topological relationships with other enterocytes in the follicle-associated epithelium of Peyer's patches of albino rat small intestine.

Materials and methods: 10 mature albino male rats weighted 200,0±20,0 g were involved into the study. Anatomical dissection with the sampling of the sections of the small intestine containing Peyer's patches was carried out with subsequent embedment of the latter into paraffin blocks and making of serial histological sections of 4 μm thick in the cross-section of the small intestine, followed with hematoxylin-eosin staining. The specimens were studied and documented on the "Konus" light microscope equipped. Morphometric characteristics of the specimen tissue structures were studied using the Sigeta X 1 mm/100 Div.x0.01mm stage micrometer.

Results: The findings of the study revealed enterocytes with phagocytic properties found in the lymphoid-associated epithelium of Peyer's patches of the small intestine of albino rats. Moreover, if they are clearly visualized at the light-optical level, then M-cells are poorly recognizable, which is consistent with a similar assessment made by other authors.

Conclusions: Given this, the issue on the topology and functional purpose of M-cells remains uncertain to date and, thereby, the prospect of further research is being outlined, which, in our opinion, can be successful using the method of stereomorphological analysis. For this purpose, multilayer plastic reconstruction methods can be used for serial semi-thin sections of Peyer's patches embedded in epoxy resin, according to the requirements of transmission electron microscopy.

KEY WORDS: lymphoid-associated epithelium, Peyer's patches, small intestine, albino rats, M-cells, phagocytic enterocytes

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INTRODUCTION

It is generally accepted that initiation of the intestine immune responses is triggered by the specialized cells within enterocytes, overlying the luminal surface of the Peyer's patches. They are commonly known as M-cells, which, unlike typical enterocytes (according to popular opinion) have a reduced cytoplasm due to the presence of a basolateral pocket, where lymphocytes, macrophages and dendritic cells are populated [1-4].

M-cells are often called immunosurveillance posts in the intestinal epithelium. Publications report that in the epithelium, overlying the Peyer's patches (follicle-associated epithelium), they account for no more than 10% [5, 6]. It is believed that due to their transcytotic activity, M-cells are able to transfer various antigens from the intestinal contents into the basolateral pocket unchanged, where they are captured by dendritic cells and macrophages that, as a result of processing, present the antigen to T-lymphocytes, thereby launching the development of immune responses [3, 5, 7-9].

THE AIM

Based on the above cytological signs of M-cells, we set the goal of more detailed clarification of some of their topological relationships with other enterocytes in the follicle-associated epithelium of Peyer's patches of albino rat small intestine.

MATERIALS AND METHODS

10 mature albino male rats weighted 200,0±20,0 g were involved into the study. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) at the Ukrainian Medical Stomatological Academy in compliance with the regulations on keeping experimental animals, adopted by the European Parliament and Council Directive (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013), (Minutes No. 178 as of 24.12.2019 of meeting the Commission on Biomedical Ethics at Ukrainian Medical Stomatological Academy) [10-12].

After vivisection made by thiopental anesthesia overdose (75 mg/kg of animal body weight intramuscularly in the upper third of the thigh of the hind paw) [13] in

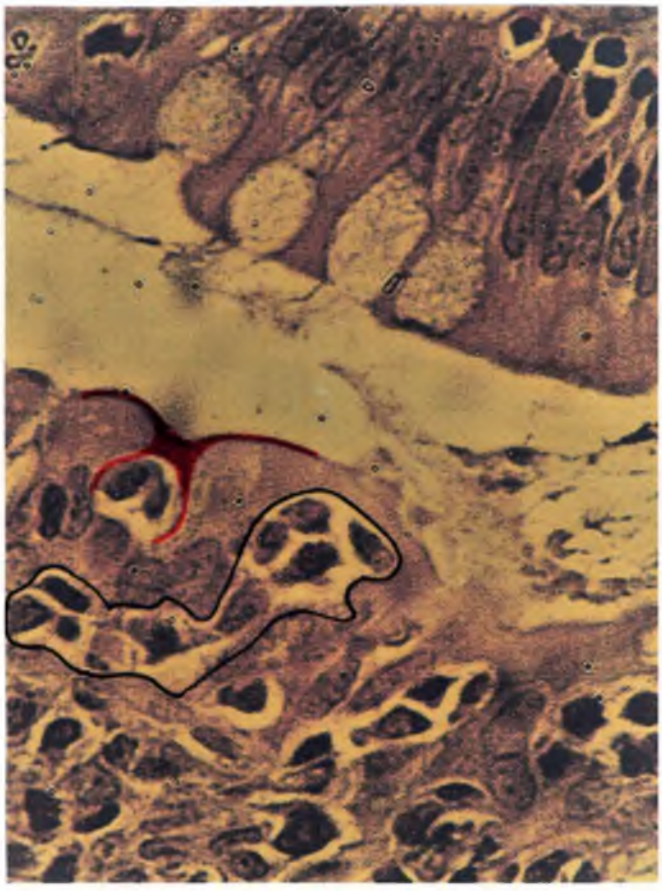

Fig. 1. Microscopic structure of the segment of lymphoid-associated epithelium of the big lymphoid nodule and adjacent intestinal villus of the Peyer's patch of the small intestine of albino rats. The black outline indicates the border of the intraepithelial lymphoid compartment, and the putative M-cell is colored red. Paraffin section; H&E stain; 100× magnification.

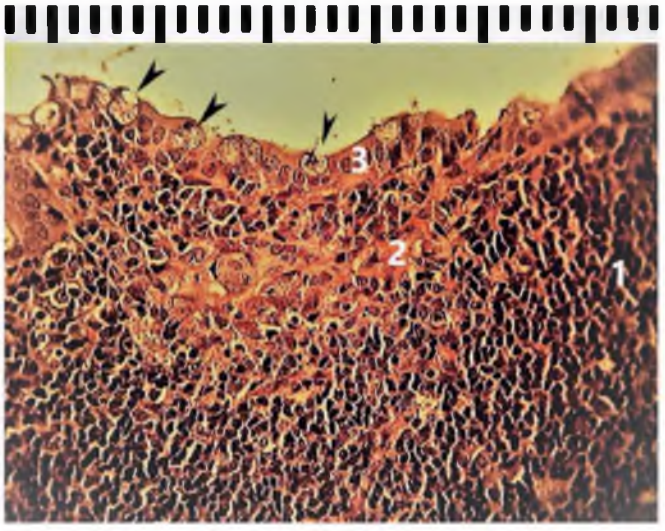

Fig. 2. The apical part of the lymphoid nodule of Peyer's patch of the small intestine. Paraffin section; H&E stain; 40× magnification (the step of the scale is equal 10 μm).
1 – lymphocytic elements; 2 – connective tissue layers; 3 – enterocytes with phagocytotic phenomena within lymphoid-associated epithelium (marked by arrows).

compliance with the requirements for dissection of the abdominal cavity, the entire complex of the gastrointestinal tract was removed, which was fixed in 10% formalin solution for two days. Following a week (after pre-washing in a running water) the murine gastrointestinal tract was examined and sections of the small intestine, containing Peyer's patches, were selectively excised. The set of Peyer's patches were clearly visualized along its length beneath the serous membrane (on the side opposite to the mesentery attachment site) in the form of whitish spots, different in shape and size.

The specimens, after washing from formalin and dehydration in alcohol of increasing concentration, were embedded into paraffin blocks, from which serial sections of 4 μm thick (Microm HM 325) were obtained in the cross-section of the small intestine and, subsequently, stained with hematoxylin-eosin. Their study and documentation was carried out using the "Konus" light microscope equipped with the Sigeta DCM-900 9.0MP digital microphoto attachment and the Biorex 3 program (serial number 5604) adapted for these studies. The morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system of visual analysis of histological specimens, as well as using the Sigeta X 1 mm/100 Div.x0.01mm stage micrometer, the scale of which (equal to 1 mm, where a small step corresponds to 10 μm) was applied to the corresponding microimage obtained in the same magnification.

RESULTS

A thorough targeted study of serial paraffin sections of Peyer's patches revealed an extremely large variety of configurational features of their epithelial overlay, which is called the follicle-associated epithelium in the literature. Noteworthy, such a name cannot be considered correct, because the word "follicle" used in it is an anachronism in relation to the formations that make up Peyer's patches. It is known, they are called lymphoid nodules. Therefore, the epithelium, overlaying them, is more correctly called the "lymphoid-associated" epithelium, which we will use hereinafter [14-18]. In our opinion, this configurational variability depends on situationally changing factors of antigenic effect, i.e., functional polymorphism is characteristic of lymphoid-associated epithelium of lymphoid nodules of Peyer's patches, usually not highlighted in the literature.

But most often, it clearly reveals the cluster pattern of distribution of enterocytes in the form of epithelial buds, which were described in the previous publications. It has been established that some of these cluster groups have the form of specific compartments, the inner contents of which are represented by small grouped aggregations of lymphocytic elements separated from the intestinal contents by a thinned layer of enterocytes (Fig. 1). At the same time, with great difficulty (as a result of multiple viewing of serial sections), it was possible to identify specifically shaped cellular elements within superficially

thinned epithelium, which are characterized by the presence of extended cytoplasmic processes, circumferencing a relatively extensive lymphoepithelial compartment. We believe that it is the hardly recognizable cellular elements that can be assigned to M-cells, since it was not possible to identify other cells corresponding to those described in the literature as part of the lymphoid-associated epithelium of Peyer's patches of the small intestine of albino rats. Of note, according to most authors, the ultimate cytological sign of M-cells is the presence of cytoplasmic invagination called a "pocket" or "niche", resided by lymphocytes together with dendritic cells and macrophages, in their basolateral part [4, 19, 20]. Consequently, the question arises: can an epithelial cell with a size of only about 15 μm have invagination of such a depth that would accommodate at least several lymphocytes, including macrophages and dendritic cells? Apparently, the answer to this question is obvious. And it should be noted that the authors, who kept to this opinion, present as an evidence arbitrary corresponding graphic drawings only, but not microphotographs, and if the latter are given, then these elements noted as M-cells are not indisputable [21].

DISCUSSION

The fact is that the lymphoid-associated epithelium of Peyer's patches is a polarized monolayer populated by various types of enterocytes, including predominant absorbing cells, as well as endocrinocytes, goblet cells and tuft cells, which are visualized mainly by electron microscopy [22-24]. Importantly, cytological differentiation of lymphoid-associated epithelium is complicated by the fact that some authors are inclined to believe that M-cells and tuft cells are the same [25].

Notably, the study revealed clear signs of plasmolemma rupture in the apical part of some enterocytes of Peyer's patches and the presence of a granular conglomerate of unknown nature (Fig. 2). This phenomenon can be interpreted in two ways: either this refers to the moment of extrusion of secretion products, for example, from goblet cells, or the process of phagocytosis by a cell of some parietal material is observed. According to our data, this phenomenon is observed in alternating order over almost the entire surface of the lymphoid-associated epithelium. If we assume the process of phagocytosis, then the question arises as to the type of cells it is carried out. By all cytological features, they cannot be identified with M-cells, which, according to existing ideas, possess such properties.

To clarify this, publications report that almost all enterocytes of lymphoid-associated epithelium are capable of phagocytosis, including goblet cells [26-28]. It is obvious that all this does not fully fit into the concept of M-cells as the only cellular structures that mediate between antigens of intestinal contents and lymphoid tissue of Peyer's patches.

CONCLUSIONS

Thus, the findings of the study revealed enterocytes with phagocytic properties found in the lymphoid-associated epithelium of Peyer's patches of the small intestine of albino rats. Moreover, if they are clearly visualized at the light-optical level, then M-cells are poorly recognizable, which is consistent with a similar assessment made by other authors.

Given this, the issue on the topology and functional purpose of M-cells remains uncertain to date and, thereby, the prospect of further research is being outlined, which, in our opinion, can be successful using the method of stereomorphological analysis. For this purpose, multilayer plastic reconstruction methods can be used for serial semi-thin sections of Peyer's patches embedded in epoxy resin, according to the requirements of transmission electron microscopy.

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The Authors declare no conflict of interest.

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