

REVIEWS

M cells – the pathway of antigen penetration into lymphoid tissue

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SUMMARY

M cells – the pathway of antigen penetration into lymphoid tissue

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The pharyngeal tonsil is the mucosa-associated lymphoid tissue (MALT) component, apart from the Peyer's patches of the ileum as well as lymphoid tissue of the bronchi and genitourinary tract wall. The mucous membranes are the site of immune response initiation due to the interaction of antigens with immune cells. M cells play a major role in the transport of antigens to MALT. Antigens present on the surface of the mucous membranes are absorbed by specialized M cells and transported in follicles in an unchanged form to the subepithelial space to be transformed by macrophages and dendritic cells, which in turn present antigens to T lymphocytes.

Key words: *M cells, mucous membrane, antigens*

Human mucosa, mainly in the respiratory and alimentary tract, is the site of interaction with an unlimited number of antigens from the external environment. The function of the epithelium that lines the mucous membranes is to provide an efficient barrier against infectious and potentially harmful agents [5]. This role can be fulfilled due to the fact that the epithelium is built up of cylindrical cells which adhere closely to one another and thus constitute a mechanical barrier for microorganisms and macromolecules; moreover, mucus and glycocalyx locally produced on the epithelial surface [33], and cilia in the respiratory tract, provide additional support to this mechanical barrier.

However, it is the immune system associated with the mucous membranes that plays a predominant

STRESZCZENIE

Komórki M - droga wnikania antygenów do tkanki limfatycznej

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Migdałek gardłowy obok kępek Peyera jelita krętego oraz tkanki chłonnej rozmieszczonej w oskrzelach i w ścianie układu moczowo-płciowego stanowi składową tkanki limfatycznej związanej z błonami śluzowymi (MALT – mucosa-associated lymphoid tissue). Błony śluzowe stanowią miejsce inicjacji odpowiedzi immunologicznej w wyniku zetknięcia antygenów z komórkami układu immunologicznego. Istotną rolę w transporcie antygenów do przestrzeni MALT odgrywają komórki M. Obecne na powierzchni błon śluzowych antygeny są wchłaniane przez wyspecjalizowane komórki M i transportowane w pęcherzykach w niezmienionej postaci do przestrzeni podnabłonkowej, gdzie ulegają przetworzeniu przez makrofagi i komórki dendrytyczne, które z kolei prezentują antygeny limfocytom T.

Słowa kluczowe: *komórki M, błony śluzowe, antygeny*

role in defense against external threats. The system works through aggregates of lymph follicles and single lymph follicles located in the mucosa and submucosa. The mucosa associated lymphoid tissue (MALT) includes gut associated lymphoid tissue (GALT), bronchus associated lymphoid tissue (BAL), nasal associated lymphoid tissue (NALT) containing palatine tonsils and pharyngeal tonsil, and the lymphoid tissue of the genitourinary tract. The lymphatic system of the mucous membranes supports the protective barrier through B-cell produced IgA which is released onto the mucosa surface [42]. T- and B-cells that are present in the secretion covering the tonsils in the proportion resembling that observed in the tonsillar tissue play a similar role,

although no correlation is noted between the percentage of cells in the secretion and tonsillar tissue [2]. *Bauer et al.* [2] indicate that migration of lymphocytes from the tonsil to the secretion is an active, selective process and that bacterial colonization of the mucosal surface and the epithelial immune reaction may stimulate migration of specific types of cells.

Many authors claim that the immune system of the mucous membranes is the site of immune response initiation [22, 36]. The response can be initiated when antigens interact with immune cells. M cells (microfolds) constitute the pathway through which antigens reach MALT [24, 30].

OCCURRENCE M CELL

M cells can be found in the mucosa of the intestines [11, 20, 34] and tonsils [11]. In the intestines, Peyer's patches form domes composed of follicle-associated epithelium (FAE) projecting above the follicles and their vicinity. The domes lack intestinal villi and possess M cells accounting for approximately 10-30% of cells in this epithelium [30, 41]. In the palatine tonsils they are located in the lymphoid epithelium and are most numerous in the apical surfaces of the crypts which correspond to the subepithelial lymphoid follicles [18], and in the pharyngeal tonsils between the ciliary epithelial cells [10].

The origin of M cells has not been fully elucidated yet. The hypotheses concerning their formation from mature or immature enterocytes are considered [7, 11, 32]. *Gebert et al.* [20] suggest that M cells constitute a separate cell line, with a significant role of B lymphocytes in their formation. However, according to *Mach et al.* [30], there are two types of M cells in the intestine – Mf cells found in the follicle-associated epithelium (FAE), whose growth is B-cell dependent, and Mv cells, B-cell independent, located on intestinal villi.

M cells adhere closely to the surrounding enterocytes by binding to them with closing junctions and desmosomes [16, 26]. In the apical part, M cells, unlike enterocytes, lack the typical brush border but instead have variable microfolds [11]. In the basolateral part, they have cytoplasmic indentations which increase the cell surface and form pockets. The pockets house a number of intraepithelial migrating cells [9], with the predominance of T and B lymphocytes in similar proportions. These are mainly TCD4⁺CD45RO⁺ memory T lymphocytes and sIgD-CD20⁺ B lymphocytes. Moreover, dendritic cells and macrophages can be found in the pockets [5, 14]. In the basal membrane, below M cells, there are macrophage-like cells that form aggregates. These cells have a light nucleus, elongated cytoplasm contain-

ing acidophilic lysosomes and remnants of the phagocytized bacteria [9, 11, 16, 20, 30]. CD11c⁺ dendritic cells present in the pockets and found close to M cells stimulate antigen transport by M cells via the production of MIF cytokine (macrophage migration-inhibitory factor) [31], and thus facilitate passage of bacteria through the mucous barrier. In the pharyngeal tonsils the percentage of dendritic myeloid CD11c⁺ cells and plasmoid CD123⁺ cells is around 0.5% [43].

STRUCTURE M CELL

The solid framework of the internal filaments built up of cytokeratin and vimentin, forming an arch around the intraepithelial pocket and a thin network around the cellular nucleus contributes to the unique structure of M cells [20, 35]. Their microfolds are irregular and contain cytoplasm devoid of microfilaments which are visible in the villi of the adherent cells. They have a well developed tubular/follicular system, small follicles in the cytoplasm of these cells have been visualized by electron microscopy [16]. Moreover, M cells, unlike enterocytes, show expression of such proteins as actin, β -catenin, E-cadherin and β -actin. *Brayden et al.* [7] claim that apart from maintaining a tight connection to the surrounding cells, these proteins take part in the process of endocytosis.

FUNCTION M CELL

The major function of M cells is to transport exogenous substances from the intestinal lumen or the respiratory tract to the lymphatic system accompanying the mucous membranes. This function can be performed thanks to microfolds, thin layer of glycocalyx, lack of activity of hydrolytic enzymes in the apical part of the cell membrane and a small number of intercellular lysosomes [19].

The transcellular transport consists of three steps: endocytosis through the cell membrane, transport of absorbed substances in follicles and exocytosis through the basolateral part of the cell membrane [11, 12, 32].

The receptors located at the apex of M cells are considered to be the antigen-binding site. They include: mucin receptor MUC2 [6], sialylated Lewis antigen A (SLAA) [21] and lectin receptor [6]. *Kyd et al.* [28] believe that the process of antigen absorption by M cells also involves $\lambda 5\beta 1$ integrin, TLR-4 and PAF receptor. IgA, which is secreted to the surface of the mucous membranes, selectively adheres to the apical part of the M cells. The significance of the interaction between IgA and M cells has not been fully elucidated. One of the hypotheses assumes that

absorption of the IgA-antigen complex exerts a modulatory effect on the adhesion of antigens to the cells possessing the receptor for the same immunoglobulin [35, 42].

The mechanism, due to which M cells absorb microorganisms and macromolecules depends on the character of this material. Large molecules and bacteria induce the process of phagocytosis accompanied by cytoskeleton rebuilding, which allows active formation of structures that resemble pseudopodia. Viruses and other adhering molecules are absorbed in the process of endocytosis by clathrin-covered vesicles, whereas non-adhering substances are internalized in the process of liquid phase endocytosis [32, 35].

In all cases, further transport of the material is fast, lasting about 10 min. [35], and the antigens do not undergo substantial ultrastructural changes [11]. However, the finding by *Finzi et al.* [15] that M cells possess enzymes, e.g. cathepsin E, characteristic of antigen-presenting cells and the presence of MHC class II on the basolateral part of the cell membrane [1] may indicate that M cells do not only transport antigens but also take active part in the early phase of the immune response induction.

The CaCo-2 cells are the best known line imitating *in vitro* human intestinal epithelium [37]. They are characterized by tight intercellular junctions, show the expression of characteristic enzymes (alkaline phosphatase) and possess transport systems (glycoprotein P, cytochrome P-450 3A4) [38].

Many *in vitro* studies are performed on the function of M cells using the cell line CaCo-2. The research concentrates, among others, on the immune response to food allergens [13, 25] and adhesion of pathogenic bacteria [3, 8, 39] and probiotic bacteria [4, 29, 39, 40] to the intestinal epithelial cells.

Since the mechanisms of bacterial and viral binding by M cells vary greatly, which has been shown in review papers by *Holmgren* and *Czerkinsky* [23] as well as *Carr et al.* [11], further studies focus on searching for the specific target ligands which by binding to M cells would allow supply of antigens to the immune system of the mucous membranes, containing approximately 70% of the human immune cells. Thus, M cells could become the site for new antigens that induce the antigen-specific immune response, which may broaden the spectrum of oral/nasal vaccines [27].

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