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Review

The role of M cells in mucosal immunity

L. J. Hathaway and J.-P. Kraehenbuhl*

Swiss Institute for Experimental Cancer Research and Institute of Biochemistry, University of Lausanne, Chemin des Boveresses 155, CH-1066 Epalinges (Switzerland), Fax + 41 21 652 69 33, e-mail: jean-pierre.kraehenbuhl@isrec.unil.ch

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Abstract. Mucosa-associated lymphoid tissue in the respiratory and digestive tracts are covered by a specialized epithelium, the follicle-associated epithelium, which includes M cells, which are specialized for the uptake and transcytosis of macromolecules and microorganisms. Following transcytosis, antigens are released to cells of the immune system in lymphoid aggregates beneath the epithelium where antigen processing and presentation and stimulation of specific B and T lymphocytes are achieved. Circulation of the lymphoid cells enables their homing to their original, and other, mucosal sites where they exert the effector function. Such a response may be dominated by secretory immunoglobulin A release and may include cytotoxic T lymphocyte action.

Binding of particles to the apical M cell membrane may be nonspecific or due to specific interaction between molecules such as integrins and lectins. Exploiting the specific binding to M cells is an aim for mucosal vaccination, for example to increase the efficiency of uptake of an oral vaccine by its conjugation to an M-cell-specific molecule. Alternatively, an M-cell-specific live vector, such as attenuated *Salmonella* bacteria, may be used to deliver epitopes of other organisms. Mucosal vaccination efficiency may also be enhanced by a temporary increase in the number of M cells.

Therefore, investigation of the properties and ontogeny of M cells must be pursued to allow the development of better mucosal vaccines for the future.

Key words. M cell; mucosa; immunity; vaccination; ontogeny.

Introduction

The human body is separated from the external environment by its covering of skin which protects from invasion by pathogens and allows the maintenance of internal homeostatic conditions. However, exchange of materials with the environment is crucial for maintenance of this homeostasis, for example respiratory gases in the lungs and nutrients in the gut. The areas where transfer occurs, the mucosae, are necessarily thin and tend to have an epithelial membrane that produces mucus at its free border. By virtue of their thin and moist nature, mucosal surfaces are more susceptible than skin to invasion by pathogens and so have a dedicated branch of the immune system for their protection. The mucosal epithelium of the oral cavity, pharynx, oesophagus, urethra and vagina are composed of a stratified squamous or stratified columnar epithelium, and antigen sampling at these sites is believed to be performed by migrating dendritic cells. However, the vast majority of mucosal epithelia are simple, consisting of a single cell layer. In particular, the gut and respiratory tracts have simple mucosal epithelia within which are some cells specialized for antigen uptake. In the gut these specialized cells are above aggregations of

* Corresponding author.

lymphoid tissue known as Peyer's patches and are known as M cells due to the microfolds on their apical surface. They are of great interest for the design of future vaccines which may be targeted to the M cells for enhanced efficiency of uptake. It is these M cells, and their role in mucosal immunity, which are the subject of this review.

Innate and adaptive immunity at mucosal surfaces

Mucosal surfaces are protected by both innate and adaptive immune systems. Innate mechanisms include the trapping of pathogens in mucus, which is enhanced by the binding of mucin to lectins on bacteria. Movement of the mucus by beating cilia in the lungs and the peristaltic movement of the gut helps to prevent attachment of pathogens to the mucosal surfaces. Other innate mechanisms to protect mucosal surfaces include the destruction of pathogens by the low pH in the stomach and by digestive enzymes in the gastrointestinal tract, and competition for nutrients from commensal organisms. Other mucosal secretions, such as tears, damage bacterial cell walls due to the action of the enzyme lysozyme which they contain. Epithelial cells are also considered to play a role in innate immunity since they can be induced to produce proinflammatory cytokines such as interleukin (IL)-8 and GROalpha in response to stimuli such as invasion by pathogens [1–3]. Such an inflammatory response may lead to the elimination of the pathogen and therefore constitute a first line of defence.

Protection at mucosal sites due to adaptive immune responses is achieved to a large extent by immunoglobulin A (IgA), the predominant antibody at these locations and by far the most abundant antibody in the body [4]. Since antibody responses to mucosally administered antigens can be detected at mucosal sites and systemically it appears that the mucosal immune system is separate from, but linked to, the systemic immune system. Systemic immunization is generally not effective in inducing immune responses at mucosal sites, but immunization via mucosal routes can induce systemic and mucosal immunity.

The mucosal immune system may be divided into inductive sites, where antigen is taken up to initiate an immune response, and effector sites, where the immune response is expressed. M cells, which are found in the epithelium overlying inductive sites, take up antigen and pass it to the cells of the immune system beneath. Following uptake by antigen-presenting cells (APCs), processing occurs and then presentation to T cells, which in turn stimulate the development of IgA-committed antigen-specific B cells. The activated antigen-specific B and T cells leave the inductive site and are carried to the draining lymph node. Eventually, the lymph drains into the blood, and the cells home to the mucosal site from which they originated and to others. The homing of the cells to different mucosal sites gives rise to the common mucosal immune system (CMIS) [5]. The homing occurs by the specific binding of molecules on the surface of the lymphocytes to molecules on the cells of high endothelial venules (HEVs) and small flat venules in mucosal tissue. Human and mouse HEV cells in the gut express mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which binds the integrin $\alpha 4\beta 7$ on the lymphocytes [6]. There appears to be some compartmentalization within the mucosal immune system, which may be due to different homing receptors and their ligands at different sites as discussed in a recent review [7]. The effector lymphocytes such as T helper (Th) cells, cytotoxic T lymphocytes (CTLs) and IgA-producing B cells therefore find their way to mucosal effector tissue in the gastrointestinal, respiratory and genital tracts and secretory glands. Lymphoid tissue at the effector sites tends to be more diffuse than at the inductive sites.

The majority of IgA released from plasma cells at mucosal effector sites is in the form of a dimer. The two IgA molecules are linked at the constant regions of their heavy chains by a peptide known as the J chain, which is also produced by the plasma cell. Mucosal epithelial cells have polymeric immunoglobulin receptors (pIgR) on their basolateral membranes [8]. The dimeric IgA binds to the pIgR, triggering its internalization and transport through the epithelial cell. The dimeric IgA is expressed on the luminal side of the epithelial cell where the pIgR is cleaved to release the antibody. Part of the pIgR remains attached to the antibody and is known as the secretory component; it may protect the antibody from enzymatic cleavage.

Secretory IgA may protect mucosae by several different mechanisms, including immune exclusion where IgA binds pathogens, preventing their attachment to epithelial cells [8]. IgA may also have a role in intracellular neutralization of virus in epithelial cells [9], and the binding of IgA to pathogens in the lamina propria may cause them to be exported out into the lumen as immune complexes [10]. Phagocytosis may be promoted as IgA binds to Fcα receptors on various cells, and this antibody may also enhance the sticking of some bacteria to mucus, neutralize toxins and interfere with growth factors required by pathogens. Of the two known subclasses of IgA discovered in humans, IgA1 predominates in the serum and upper aerodigestive tract, whereas IgA2 predominates in the normal large bowel mucosa [11].

Cell-mediated cytotoxicity, antibody-dependent cytotoxicity involving IgA and natural killer cell activity as well as functional CTLs have all been found in mucosa-

associated tissue and may therefore have roles in mucosa effector sites [12, 13].

Once an antigen has been taken up across a mucosal surface, rather than inducing a protective immune response, it may induce 'oral tolerance'. This is where mucosal administration of an antigen induces peripheral tolerance to subsequent systemic exposure to the same antigen. Without this the body would mount an immune response to every foreign antigen taken up across the mucosa, of which there will be many, particularly in the gut. M cells are specialized for the uptake of particulate antigens which they then pass to APCs at the mucosal inductive sites; but soluble antigen may enter the intestinal enterocytes and not reach an inductive site. Thus, the result of uptake of a soluble antigen may be tolerance, whereas a particulate antigen may induce an immune response [14].

Inductive sites of the mucosal immune system

At areas where antigen is taken up by mucosal tissue are inductive sites specialized for the first steps of an immune response. These consist of organized aggregates of lymphoid cells known as organized mucosa-associated lymphoid tissue or O-MALT. Mucosae of different regions of the body have their own version of MALT, such as gut-associated lymphoid tissue (GALT), bronchial-associated lymphoid tissue (BALT) and nasal-associated lymphoid tissue (NALT) (table 1). The various MALTs have a similar, but not identical, cellular composition: NALT has a greater proportion of T cells than Peyer's patches (PPs), and of these a greater percentage are CD4+ as compared with CD8+ T cells. NALT T-cell populations resemble those of the spleen rather than the PP, but NALT and PP, contain similar numbers of mature IgM+ IgA− B cells [15]. A degree of compartmentalization leads, for example, to the majority of lymphocytes stimulated in GALT to migrate back to gut lamina propria [11]. GALT includes PPs, the appendix and solitary lymphoid nodules [12]. PPs, first described by J. C. Peyer in 1677 [20], are lymphoid follicles in the wall of the small intestine which project into the lumen, forming dome shapes. The follicles contain B and T lymphocytes as well as the specialized APCs, dendritic cells and macrophages. The epithelium overlying the PP dome is called the follicle-associated epithelium (FAE) and contains the M cells specialized for the uptake of antigen. M cells have also been observed in the epithelium of other MALTs with a morphology similar to that of GALT M cells, for example in the palatine tonsils of rabbits [21].

Characteristics of M cells

The morphology of human M cells was first described by Owen and Jones [22], who found that rather than having the closely-packed microvilli characteristic of small intestinal epithelial cells, some cells in the epithelium over PPs have microfolds. The M cells also have prominent Golgi complexes and many mitochondria surrounded by rough endoplasmic reticulum. They are linked to adjacent cells via desmosomes and interdigitations. The lack of a brush border of microvilli in these cells correlates with the redistribution of villin from the apical membrane to the cytoplasm and reflects the reorganization of the F-actin network [23]. In addition, changes in cytoskeleton in these M cells compared with enterocytes were shown by the fact that pig M cells, but not enterocytes, were labelled intensely with anti-cytokeratin 18 antibody, but cytokeratin 19 was more abundant in enterocytes than M cells [24]. M cells are surrounded by microvilli-bearing cells and contain vesi-

Table 1. A comparison of GALT, NALT and BALT (in mice unless otherwise stated).

Mucosa-associated lymphoid tissue	Location	Cellular composition	Presence of M cell in epithelium
Peyer's patches (GALT) [15]	Intestine wall	28% T cells CD4:CD8 ratio 3.7:1 70% B cells 2% macrophages	yes
NALT [15]	Entrance of nasopharyngeal duct	40% T cells CD4:CD8 ratio 10:1 55% B cells 4.5% macrophages	yes in rats [16]
BALT [17]	At junctions between bronchi and bronchioli or between the bronchus and an artery	T cells CD4>CD8 B cells macrophages	controversial in rabbits [18, 19]

Table 2. Characteristics of M cells from various species and locations.

M cell characteristics	Species/location
Lack of brush border of microvilli, presence of microfolds	general [22]
Large invagination of basolateral membrane containing lymphocytes	general [22]
Increase in cytokeratin 18, reduction in cytokeratin 19 compared with enterocytes	pig [24]
Apical expression of digestive enzymes (e.g. alkaline phosphatase and sucrase isomaltase) reduced	general [25]
Lack of expression of polymeric immunoglobulin receptor (pIgR)	human [26]
Lower membrane potential than enterocytes	mouse [27]
Binding to Ulex europaeus agglutinin 1 (UEA-1) lectin	mouse [28]
	PP but not caecal FAE [29]
HLA-DR+ ICAM-1−	small intestine [30]
HLA-DR− ICAM-1+	colon [30]
Expression of sialyl Lewis A antigen (also on some FAE enterocytes and in goblet cell mucins)	human [32]
Binding to UEA-1, *Lotus tetragonolobus* (LTA), *Helix pomatia* (HPA), *Vicia villosa* (VVA)	rabbit tonsil [34]
Vimentin	rabbit gut and tonsils [35]
	not in mouse [23]

cles within their cytoplasm. M cells have a large invagination of the basal membrane forming a pocket in which lymphoid cells are found. A narrow layer (0.3 μm thick) of the M cell cytoplasm is therefore all that separates the lymphoid cells from the lumen, whilst the integrity of the epithelium is preserved by the tight junctions between M cells and adjacent cells [22]. Mouse M cells have a shape comparable to that of human M cells and contain fewer lysosomes and have fewer and shorter microvilli than adjacent absorptive cells. M cells outside the gut, such as in rabbit palatine tonsil epithelium, also have this characteristic shape with an invagination of the basolateral membrane forming a pocket containing lymphocytes. Gebert [21] found that the region of the basolateral membrane forming the pocket had a different lectin-binding pattern than the rest of the basolateral membrane, suggesting that the compostion of the membrane is not uniform. Apical alkaline phosphatase is less abundant, but esterase is more abundant in M cells than that of adjacent absorptive cells [25]. In contrast to villus enterocytes, human FAE does not express the pIgR and so does not appear to transport secretory IgA to the gut lumen [26]. In addition, M cells have a lower membrane potential than differentiated enterocytes, which may aid their endocytosis of macromolecules [27]. A further difference between M cells and absorptive enterocytes is the lectins which they can bind to their apical surfaces; for example the α-L-fucose-specific lectin *Ulex europaeus* agglutinin 1 (UEA-1) binds specifically to mouse PP M cells [28]. However, the identification of M cells by their lectin-binding pattern is complicated by the fact that within an individual, the lectins bound by M cells vary along the length of the intestine. Clark et al. [29] found a difference in surface glycoconjugate expression between caecal and PP M cells in the mouse with staining by UEA-1 of PP M cells but not caecal patch FAE. Ueki et al. [30] found differences between M cells from PPs of the small intestine and colon in humans: small intestinal M cells were human leukocyte antigen-D related (HLA-DR)-positive, ICAM-1-negative, whereas colonic M cells were HLA-DR-negative and ICAM-1-positive. ICAM-1 was found predominantly at the apical membrane but was not found on adjacent cells of the FAE. Also, there is variation between species; for example caecal rabbit M cells differ from those of other species in their lectin binding [31], and mouse M cells are exclusively labelled by fucose-specific lectins. In humans the M-cell glycosylation patterns are distinct from other species and preferentially display the sialyl Lewis A antigen [32]. However, this carbohydrate epitope is not a unique marker for M cells, because it is also expressed on some enterocytes of the FAE and in goblet-cell mucins. In addition, FAE enterocytes and goblet cells have lectin-binding characteristics which differ from those of the villus enterocytes and goblet cells, particularly in humans [33]. As well as M cells in the gut, lectin-binding properties of M cells in rabbit tonsil crypts have been described [34]. M cells selectively bound UEA-1, *Lotus tetragonolobus* (LTA), *Helix pomatia* (HPA) and *Vicia villosa* (VVA) lectins, whereas the other epithelial cells bound *Ricinus communis* (RCA-1) and *Arachis hypogaea* (PNA). Both gut and tonsillar M cells in the rabbit may also be identified by antibody to vimentin [35], but vimentin does not appear to be present in mouse M cells [23]. Some characteristics of M cells are summarized in table 2.

This lectin-binding ability of M cells may reflect the situation in vivo as lectin-bearing microorganisms such as *Escherichia coli* RDEC-1 [36], *Salmonella typhimurium* [37] and *Yersinia enterocolitica* [38] bind specifically to M cells. *Yersinia pseudotuberculosis* may target M cells by the binding of its invasin to β1-integrin, which can be expressed on the apical membrane of M cells, but which on other epithelial cells is restricted to the basolateral membrane [39]. Lectins bound to M cells may be rapidly endocytosed and transcytosed [28], which demonstrates a mechanism by which pathogenic microorganisms may gain entry across the epithelium by binding to M cells via lectins.

M-cell-dependent immune response induction

M cells are specialized for the uptake of antigens, their transcytosis and release to cells of the immune system in the lymphoid tissue of the mucosae. Even inert particles have been shown to be taken up from the intestinal lumen specifically by M cells. Pappo and Ermak [40] showed this when they administered fluorescent 600–750-nm latex microparticles into intestinal loops of rabbits and, by microscopy, followed the passage of the microparticles with time. No particles were found to be taken up by the villi but, 10 min after administration of the microparticles, 95% were at the FAE surface. By 90 min post-administration, some microparticles had moved through the FAE and been released into the lymphoid area of the dome. By the use of monoclonal antibodies, the cells which had taken up the latex microparticles were identified as M cells.

Cationized ferritin (CF) has also been used to investigate uptake and transport by M cells in comparison with absorptive enterocytes [41]. Bye et al. [42] allowed 60 min of exposure to CF and then assessed the abundance of CF in endocytic vesicles of absorptive enterocytes and mature and immature M cells of mice. Only 15% of absorptive enterocytes had endocytic vesicles containing CF, whereas 80% of mature M cells and 58% of immature M cells had. M cells also had greater quantities of CF internalized, and only mature M cells transported CF across the cells. Immature M cells lack a pocket and often have more free ribosomes than mature M cells and an apical surface which resembles undifferentiated crypt cells.

Since such inert substances as latex microparticles and cationized ferritin are taken up by M cells, this indicates that specific receptor binding is not required for uptake. However, it appears that some bacteria and viruses do target M cells, presumably to exploit their transcytotic activity for invasion of the body. For example, *Salmonella typhi* bacteria pass through M cells to cause a systemic disease [37], reoviruses are transported through M cells to cause pathogenesis in mice [43] and human immunodeficiency virus-1 (HIV-1) is endocytosed and transcytosed by M cells of mice and rabbits [44]. Entry of bacteria generally considered as noninvasive, such as *Vibrio cholerae*, via M cells has been investigated in rabbits. Owen et al. [45] inoculated vibrios into the intestinal lumen and observed by transmission electron microscopy that they were phagocytosed by M cells into vesicles which were released from the basolateral membrane to the underlying lymphocytes and macrophages of the PPs. Uptake of *V. cholerae* by epithelial cells other than M cells was not observed. No uptake of vibrios killed with acid, heat, formalin or ultraviolet (UV) irradiation was observed, perhaps due to the alteration of an M cell binding ligand on the vibrio surface by such treatments or due to the reduction of their motility. The ability of M cells to take up particles may, in part, be determined by their lack of a terminal web of microfilaments as well as their lack of an organized brush border of microvilli and glycocalyx. In addition, Owen et al. found that there was a paucity of mucus over M cells relative to that over absorptive enterocytes, thus increasing the accessibility of M cells to lumen antigens.

Antigens tend to be transcytosed through M cells and released into the pocket beneath intact [46], and so M cells are not thought to have a major role in the processing and presentation of antigen. However, acidic lysosomal-like vesicles in the cytoplasm and major histocompatibility complex (MHC) class II expression on the basolateral membrane was found in a study of rat intestinal M cells which would be consistent with a role in antigen processing and presentation [47]. The aspartic proteinase cathepsin E has been localized to M cells of humans and rats, which may enable M cells to contribute to the processing of macromolecules and microorganisms being transported by them to the lymphoid cells beneath the epithelium [48].

It has been suggested that M cells may aid the immune response induction to the antigen they are transporting by releasing a costimulatory signal for T and B cell proliferation in GALT. Pappo and Mahlman [49] isolated M cells from rabbit FAE by flow cytometry using a monoclonal antibody which recognizes an M cell pocket-domain-restricted surface molecule. The M cells secreted IL-1, as assessed by the ability of their culture supernatant to induce proliferation of a T cell clone, and this secretion was increased when they were stimulated with lipopolysaccharide (LPS). Antibodies against IL-1 inhibited the T cell proliferation in response to the M cell supernatant. Therefore, in vivo as M cells transcytose bacteria from the lumen to the pocket, LPS may stimulate them to release IL-1 aiding proliferation of the lymphocytes for the successful induction of the first step of a mucosal immune response. There are more T cells at the FAE than the villi epithelium, and the T cells appear to be clustered adjacent to M cells. Since the ratio of CD4+ to CD8+ T cells is also greater at the FAE than the villi epithelium, the FAE may be associated with induction of helper T cells [50]. These T cells may help both a protective secretory IgA response and a local CTL response to antigen taken up by M cells [51].

Mucosal vaccination

Since the majority of pathogens enter the body by crossing mucosal surfaces, there is great interest in developing vaccines which induce protective immune

responses at mucosae as well as systemically. The most successful mucosal vaccine used for humans to date is the oral (Sabin) poliomyelitis vaccine, which consists of three live attenuated strains of the poliomyelitis virus [52]. Another mucosal vaccine which has been used to prevent disease in humans is the Ty21a strain of *Salmonella typhi* bacteria, which protected against typhoid when administered orally [53]. *Salmonella* can be used as a vector to carry foreign antigens [54] and binds selectively to M cells [55]. An alternative approach to the use of live vectors for mucosal vaccination is the use of subunit vaccines which avoid the risk of reversion to virulence of the delivery vector but which tend to be less immunogenic and may therefore need to be administered with an adjuvant. The B subunit of cholera toxin (CTB) has been proposed as a protein with unique mucosal adjuvant properties which could be exploited to enhance the uptake of mucosally administered antigens. Frey et al. [56] investigated the uptake of CTB coupled to fluorescein isothiocyanate (FITC) representing a soluble antigen, and CTB coupled to gold particles, final diameter 1.13 μm, representing bacteria. CTB coupled to FITC and CTB-coated fluorescent microparticles were administered to ligated loops in rabbits, and the CTB-gold particles were applied to mucosal explants ex vivo. CTB can bind ganglioside GM1 glycolipid on the apical membrane of all intestinal epithelial cells, and the soluble antigen CTB-FITC was found to bind to absorptive enterocytes and M cells. In contrast, the CTB-gold conjugates adhered only to M cells, whereas the CTB-coated microparticles did not adhere to any of the epithelial cells. Frey et al. explained this by the fact that M cells have a relatively thin glycocalyx which allows soluble antigens and 28.8-nm-diameter particles to cross. The thicker glycocalyx of absorptive enterocytes appears to allow soluble antigens to cross but not particles of 28.8-nm diameter. However, M cells seemed to have a thick enough layer to prevent particles greater than 1 μm from binding to them. Therefore, attaching CTB to a vaccine would only be useful if the antigen was below a certain size. To protect antigens from digestion by gut enzymes, they may be encapsulated within liposomes, thus also making them particulate. Cholera toxin (CT) and its B subunit have been coupled to liposomes to combine the advantages of antigen protection with increased uptake. Harokopakis et al. [57] have described a method for this coupling which maintains the biological activity and immunogenicity of the CTB which was used to immunize rats intragastrically. Liposomes have also been coated with antigen-antibody complexes, irrelevant to the antigen, within the liposomes to exploit the ability of M cells to recognize antigen-antibody complexes. Velez et al. [58] administered such liposomes to mice intrajejunally and found that they induced greater mucosal IgA responses than uncoated liposomes. Zhou et al. [59] combined these methods of enhancing a mucosal immune response by immunizing mice rectally with antigen encapsulated in liposomes coated with IgA and administered with cholera toxin as an adjuvant. The IgA on the surface of the liposomes increased their uptake into PP mucosa. Antigen may also be protected for mucosal delivery by its encapsulation in poly(DL-lactide-co-glycolide) microparticles; for example Jones et al. [60] used this polymer to encapsulate DNA for oral administration.

Secretory IgA was found by Kato [61] in the glycocalyx and on microfolds of M cells rather than adjacent absorptive cells, and when Weltzin et al. [62] injected labelled IgA into intestinal loops, it bound to M cell luminal membranes and was transported across the M cells in vesicles. Therefore, attempts have been made to exploit the selective adherence of secretory IgA to M cells by using it as a vaccine delivery vector for foreign epitopes. Corthésy et al. [63] inserted a *Shigella flexneri* IpaB invasin epitope into rabbit secretory component (SC) and then reassociated the SC to IgA and used the construct to immunize mice orally. Antibodies to IpaB invasin were induced in serum and saliva.

Uptake of fluorescent polystyrene microspheres by M cells was increased at least threefold when the microspheres were conjugated to an anti-M-cell monoclonal antibody and administered into intestinal loops in rabbits [64]. In mice, coating of latex microspheres with *Ulex europaeus* 1 lectin enhanced their binding to M cells by 100-fold while not affecting binding to enterocytes [65]. If such an M cell-specific factor for human M cells could be coated onto particles, vaccine uptake efficiency might be greatly increased.

Ontogeny of M cells

In addition to targeting vaccines to M cells, future approaches to mucosal vaccination may include induction of a temporary increase in the number of M cells in order to increase vaccine uptake. Differentiation into M cells is incompletely understood, whereas differentiation into absorptive enterocytes has been more extensively studied [66]. Intestinal epithelial cells originate in the crypts from undifferentiated stem cells. An individual crypt situated between a villus and a PP dome may contribute epithelial cells to the villus from the villus side of the crypt and to the FAE of the dome from the dome side of the crypt. As cells migrate from the crypt up the villus, they acquire the phenotype of differentiated enterocytes. This differentiation is achieved by the transcription of genes encoding proteins required for the functions of absorptive enterocytes, including digestive enzymes such as sucrase-isomaltase and trans-

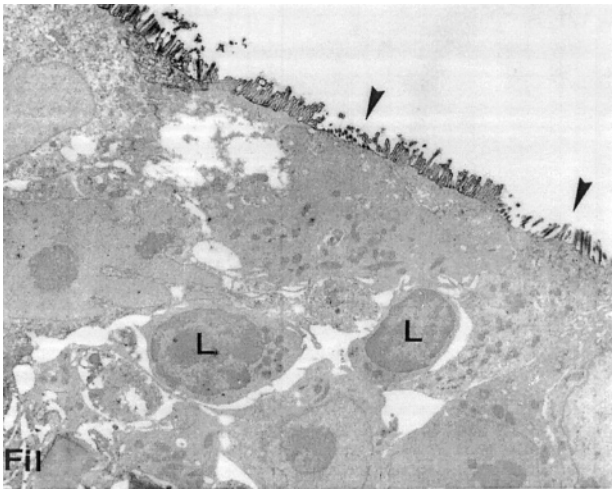

Figure 1. Transmission electron microscopy view of M cell induced by coculture of PP-derived lymphocytes with a monolayer of the Caco-2 epithelial cell in vitro (L, lymphocytes; Fil, filter). Reprinted with permission from Kerneis S., Bogdanova A., Kraehenbuhl J.-P. and Pringault E. (1997) Science 277: 949–952. © 1997 American Association for the Advancement of Science.

Figure 2. Transmission electron microscopy view of M cell induced by injection of PP lymphocytes into duodenal mucosa of a mouse. Reprinted with permission from Kerneis S., Bogdanova A., Kraehenbuhl J.-P. and Pringault E. (1997) Science 277: 949–952. © 1997 American Association for the Advancement of Science.

porters of nutrients. The pathway of differentiation of M cells is less clear. FAE cells are also derived from the crypts, but whether the M cells differentiate from absorptive enterocytes on the dome or are a separate cell lineage coming directly from the crypt is controversial. Gebert et al. [67] favoured the latter hypothesis in their recent paper describing two different types of crypts: one specialized to produce epithelial cells for domes which appeared to produce M cells, and ordinary crypts which did not produce M cells. M cell precursors were found on the sides of dome-associated crypts by labelling lectin, and cells with M cell morphology were found in strips leading from the crypt onto the dome. Gebert et al. concluded that M cells represent a separate cell line deriving directly from undifferentiated crypt stem cells. However, other studies have indicated that differentiated absorptive enterocytes can be induced to convert into M cells following contact with lymphocytes. FAE forms above aggregates of lymphoid tissue, and lymphocytes, as well as professional APCs, nestle into the pocket formed in the basolateral membrane of M cells. Evidence for the role of lymphocytes in M cell formation came from the in vitro model used by Kerneis et al. [68] in which Caco-2 epithelial cells were grown on a membrane with micropores until they formed a tight monolayer. PP-derived lymphocytes were then applied to the basolateral side of the monolayer. The lymphocytes crossed the membrane and became closely associated with the epithelial cells which acquired an M-cell-like morphology (fig. 1) and the ability to transcytose fluorescently labelled latex beads. In addition, when lymphocytes from PPs were injected into the intestinal wall of mice, M cells appeared in the epithelium over the lymphoid cells [68] (fig. 2). However, Gebert et al. [67] found that holes in the dome basal membrane, corresponding to sites where lymphocytes had crossed, were evenly distributed over the dome, in contrast to the striped distribution of M cells. They took this to indicate that it was not interaction with the lymphocytes which caused formation of the M cells. Nonetheless, the M cell phenotype does appear to be extremely plastic since a continuum of phenotypes between differentiated enterocytes and M cells has been observed [69], and phenotypically mature M cells are found predominantly in a ring on the dome with very few at the dome apex. Since apoptosis of cells from the dome occurs almost exclusively at its apex, and in chickens and mice M cells exhibited no signs of apoptosis, then it appears that in vivo the M cells (re)gain a differentiated enterocyte phenotype on their journey to the apex [70, and Sierro et al., unpublished]. The necessity of a signal, which may come from lymphocytes, is also suggested by studies with immunodeficient mice [71, and Debard et al., unpublished]. In RAG-deficient mice no PPs develop, but in B- or T-cell-deficient mice a few small PPs with FAE do

develop. It is also known that exposure to some bacteria such as *Streptococcus pneumoniae* R36a [72] and inflammation [73] can increase the number of M cells, showing that this phenotype is inducible. Oral infection of germ-free mice with *Salmonella typhimurium* aro A^- bacteria increased the number of M cells by two- to threefold [74]. Therefore, M cells may derive only from specialized crypts with only certain stem cells able to give rise to M cell precursors, but this formation of M cell precursors may require a signal which can be provided by lymphocytes. Perhaps undifferentiated Caco-2 cells are equivalent to stem cells from dome-associated crypts which give rise to M cells. Without a lymphocyte-derived signal, the Caco-2 cells differentiate into absorptive enterocytes, but when cocultured with lymphocytes, they receive the necessary signal to fulfil their destiny as M cells.

The mechanisms by which M cells are formed are currently being actively investigated. If their differentiation were fully understood, it might be possible in the future to manipulate the number of M cells to increase mucosal vaccine efficiency.

Conclusion

As the understanding of M cell features, functions and induction increases, we move closer to being able to manipulate the uptake of mucosally administered vaccines and drugs by these cells. The identification of an M-cell-specific marker would aid their study and if apically expressed may be a target for mucosal vaccines.

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