Current Concepts in Mucosal Immunity
V. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system

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Neutra, Marian R. Current Concepts in Mucosal Immunity. V. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G785–G791, 1998.—Specialized epithelial M cells, a phenotype that occurs only in the epithelium over organized lymphoid follicles, deliver samples of foreign material by transepithelial transport from the lumen to organized lymphoid tissues within the mucosa of the small and large intestines. Mounting evidence indicates that a complex interplay of mucosal lymphoid cells and luminal microorganisms with epithelial cells underlies differentiation of the M cell phenotype. The cellular and molecular features of M cells that promote adherence and transport of antigens and microorganisms are crucial for the design of mucosal vaccines and for understanding the strategies that pathogens use to exploit this pathway.

THE EPITHELIUM of the small and large intestines provides an effective barrier against the myriad foreign macromolecules and microorganisms of the gut lumen. It is remarkable that this vast cellular barrier is generally able to exclude potentially harmful and antigenic materials, given that it consists of a delicate monolayer of cells actively engaged in digestion and absorption of nutrients. The ability of the epithelium to face the outside world is enhanced by the noncellular defenses produced and/or exported by the epithelial cells and associated closely with mucosal surfaces, such as mucins (35), defensins (43), and secretory antibodies, especially IgA (39). Cells of the entire intestinal epithelium are joined by tight junctions that allow passage of ions and water but exclude peptides and macromolecules with antigenic potential (31). In addition, the enterocytes themselves are remarkably well defended by the unique structural specializations of their apical surfaces.

Enterocyte apical surfaces are covered by rigid, closely packed microvilli, the tips of which contain integral membrane mucinlike glycoproteins that form a continuous, filamentous brush-border glycoalx (32). This thick (400–500 nm) layer contains adsorbed pancreatic enzymes and stalked intramembrane glycoprotein enzymes responsible for terminal digestion and serves as a diffusion barrier that prevents direct contact of most macromolecular aggregates, particles, viruses, and bacteria with the microvillus membrane (22). The glycoalyx thus serves a protective function, preventing the uptake of antigens and pathogens while providing a highly degradative microenvironment that promotes the digestion and absorption of nutrients. The small membrane domains between microvilli that can participate in endocytosis are sequestered under the glycoalyx. Most proteins that are taken up by enterocytes are transported to lysosomes, and this tends to discourage transepithelial transport of intact antigens. Nevertheless, enterocytes can transcytose small amounts of intact proteins and peptides, and such uptake may be immunologically significant (33). The immunological importance of enterocyte transport of antigens is suggested by the interaction of intraepithelial lymphocytes with enterocytes via basolateral adhesion molecules, by the clustering of major histocompatibility class II+ macrophages (MHC II) immediately under the epithelium, and by the fact that enterocytes can serve as antigen-presenting cells in vitro. On the other hand, the diffuse lymphoid tissue of the lamina propria in vivo is not designed for efficient induction of mucosal immune responses. It has been proposed that in the gut, antigens resulting from digestion in the lumen and transepithelially transported or degraded intracellularly in enterocyte lysosomes could associate with MHC II and be presented to lymphocytes to suppress the systemic immune response to luminal antigens such as digested food products (33).

At sites of organized mucosal lymphoid follicles, in contrast, the collaboration of epithelial cells with antigen-presenting and lymphoid cells is highly developed. In the follicle-associated epithelium (FAE), specialized epithelial M cells deliver samples of foreign material through active transepithelial vesicular transport from the lumen directly to intraepithelial lymphoid cells and to organized mucosal lymphoid tissues that are designed to process antigens and initiate mucosal immune responses. The M cell transport system appears
to be key to the pathogenesis of certain bacterial and viral diseases (36, 51) and for the effectiveness of mucosal vaccines (37).

**M CELLS AS GATEWAYS TO THE MUCOSAL IMMUNE SYSTEM**

M cells provide local, functional openings in the epithelial barrier through vesicular transport activity (41). Restriction of M cells to the sites directly over lymphoid follicles seems to be due to the inductive influence of cells and/or secreted factors from the organized lymphoid tissues on epithelial differentiation, as discussed below. This arrangement serves to reduce the inherent risk of transporting foreign material and microbes across the epithelial barrier by assuring immediate exposure to phagocytes and antigen-presenting cells. Although the phenotypes of the cells in the M cell pocket have been explored by immunocytochemistry in rodents (15), rabbits (16), and humans (19), there is no direct information available concerning the interactions and events that occur in the M cell pocket.

In all species studied, the pocket contains B and T lymphocytes along with a small number of macrophages. The M cell-associated T cells are distinct from villus intestinal epithelial cells: they are mostly CD4+ and do not display the γδ T cell receptor, although in the rabbit there are cells lacking both CD4 and CD8 (16). Human M cell-associated T cells display the marker antigen CD45RO typical of activated or memory cells (19). The B cells in the pocket express IgM but not IgG or IgA, suggesting that B memory cells and/or initial B cell differentiation may occur here. The presence of memory phenotypes suggests that cells in the pocket have positioned themselves for reexposure to incoming antigen. It has been suggested that B lymphoblast traffic into the M cell pocket may allow for repeated antigen exposure and extension and diversification of the immune response (19). However, immediately under the FAE, there is an abundance of B lymphoblasts, helper T cells, and antigen-presenting cells that are presumably sufficient for initiating an immune response. What then is the role of the lymphoid cells within the M cell pocket? One possibility is that the cells in the pocket can interact very early with incoming antigens, in an environment that is sequestered from the modulating influence of systemic humoral immunity. Indeed, ultrastructural studies have shown that injected immunoglobulins do not freely diffuse into the organized mucosal lymphoid tissues (2), and we have observed that after intravenous injection, horseradish peroxidase does not readily enter the M cell pockets.

Lectin staining of M cell basolateral membranes revealed that M cells have basal processes that extend into the underlying lymphoid tissue (25). It is not known whether these extensions make direct contact with lymphoid or antigen-presenting cells in the subepithelial tissue or what role they might play. Below the FAE is the so-called “dome” region that caps the underlying lymphoid follicle. The dome contains an extensive network of dendritic cells and macrophages intermingled with CD4+ T cells and B cells that appear to be derived from the underlying follicle (15, 19). These subepithelial cell populations reinforce the idea that this is an immune-inductive site, where endocytosis and killing of incoming pathogens as well as processing, presentation, and perhaps storage of antigens occur.

There is very little information available about the actual fates of specific antigens and pathogens immediately after M cell transport. M cell-transported lectins, cholera toxin conjugates, protein tracers, and particles have been detected in cells of the dome and follicle, but whether these cells were macrophages, dendritic cells, or both was not determined. Ultrastructural studies have shown uptake of microorganisms by subepithelial cells resembling macrophages, and a light microscopic study detected Salmonella in dendritic cells of the dome region (27). Invasive pathogens such as reovirus and Salmonella would be expected to infect their specific target cells soon after entry, but further investigation is needed to determine exactly how and where nonliving macromolecules, particles, killed microbes, and specific vaccines are processed in this tissue.

**DISTINGUISHING FEATURES OF M CELLS**

The diverse phenotypes of the intestinal epithelium were originally defined by morphological criteria. For most of the cell types, specific protein markers were subsequently identified by cell fractionation and immunohistochimistry to facilitate the investigation of epithelial lineages. For example, the relative ease of brush-border isolation and analysis allowed marker enzymes to serve as hallmarks of the enterocyte phenotype. As for dispersed cell types such as goblet cells, enteroendocrine cells, and Paneth cells, the storage of secretory products, such as mucus, hormones, lysozyme, and defensins provided marker proteins that have become the standards for cell identification. Identifying cell type-specific markers for M cells has proven more difficult, because this rare cell type has not been isolated in sufficient quantities for biochemical or genetic analysis, and M cells have no stored secretory product. Thus we do not yet have a protein or gene that can serve to definitively identify M cells in all species, and for most purposes, morphological criteria continue to be used. Nevertheless, other biochemical and functional features aid in the identification of M cells.

The apical surfaces of M cells are distinguished ultrastructurally by the absence of a typical brush border and the presence of variable microvilli or microvilli folds (44) and large intermicrovillar endocytic domains (40). The cardinal feature of this cell type, however, is the unique intraepithelial “pocket.” This structural modification, the hallmark of fully differentiated M cells, provides a docking site for lymphocytes and shortens the distance that transcytotic vesicles must travel from the apical to the basolateral surface. The basolateral surface of the M cell includes the two major subdomains typical of all epithelial cells: the lateral subdomain is involved in cell-cell adhesion and contains Na+-K+-ATPase, and the basal subdomain inter-
acts with the extracellular matrix and basal lamina. The additional subdomain that lines the pocket presumably has adhesion molecules that interact with lymphocytes, but these are yet unexplored. The unusual shape of the M cell appears to be maintained by a dense scaffold of intermediate filaments, which forms an arch around the pocket and a thick network around the nucleus. In rabbits and perhaps other species, the intermediate filament protein vimentin is expressed by M cells but not other epithelial cell lineages (24). Although vimentin can be expressed by enterocytes in culture, it remains a useful marker for M cells in vivo.

The M cell apical surface differs from that of intestinal absorptive cells in other respects. Most M cells in Peyer’s patches lack the highly organized brush border with uniform, closely packed microvilli typical of enterocytes. The actin-associated protein villin, confined to microvilli in enterocytes, is diffusely distributed in M cells (29), reflecting the modified apical organization and perhaps the ability to rapidly respond to adherence of microorganisms with ruffling and phagocytosis. M cells are also recognized by their lack of certain enterocyte surface glycoproteins. Whereas enterocyte brush borders have abundant hydrolytic enzymes, these enzymes are usually reduced or absent on M cells (45, 49). In addition, the thick filamentous glyocalyx typical of enterocytes is often absent from M cells, rendering the M cell surface more accessible to luminal materials, as discussed below.

Nevertheless, M cell apical surfaces are coated with glycoproteins that display glycosylation patterns different from their enterocyte neighbors, and although the protein backbones have not been identified, the carbohydrate epitopes can be useful M cell identifiers. For example, in Peyer’s patches of BALB/c mice, lectins that recognize certain carbohydrate structures containing \( \alpha-(1–2)-fucose \) selectively stained all M cells in the FAE (9, 17, 25). Giannasca et al. (25) observed these lectin binding sites on M cell plasma membranes, including the basolateral membrane and pocket domain, as well as intracellular vesicles. Furthermore, lectins and antioligosaccharide antibodies revealed variations in the glycosylation patterns of individual M cells within a single FAE, a phenomenon that might allow the M cells to “sample” a variety of microorganisms (25, 36). However, glycoconjugates expressed on M cells in different intestinal regions and in different species are distinct (7, 23, 36), and so far there is no single, universal lectin that can serve to identify M cells.

**INDUCTION OF THE M CELL PHENOTYPE**

Differentiation of FAE and M cells involves a complex interaction between the luminal microbial flora and lymphoid cells with epithelial cells. The fact that microorganisms play a crucial role in the development of organized mucosal lymphoid tissues was first demonstrated in studies using germ-free mice. Mice reared in a germ-free environment have a reduced number of Peyer’s patches, but lymphoid follicles and M cells rapidly increase in number after transfer to a normal gauge environment (52) or after exposure to a single bacterial species (5). From these observations it could not be determined to what extent the microorganisms acted via lymphoid mediators or directly on epithelial cells. In response to adherence or invasion of pathogens, epithelial cells release cytokines and chemokines (28) that can attract local inflammatory cells and recruit lymphoid and other cells from the blood. These signaling mechanisms could play an indirect role in FAE differentiation by promoting the assembly of lymphoid follicles. Several lines of evidence indicate that cells of the mucosal follicles play an inductive role in the differentiation of the specialized FAE and M cells. However, the “natural history” of a given epithelial-lymphoid complex over the course of time has been largely unexplored.

M cells are one of several distinct phenotypes that arise from undifferentiated stem cells in the intestinal crypts, but this phenotype and other FAE phenotypes appear only in crypts adjacent to mucosal lymphoid follicles. Evidence from chimeric animals has established that in adult small intestine, each crypt is a clonal unit that produces multiple cell types that migrate upward in columns onto several adjacent villi (26). Similarly, the FAE is derived from the “follicle-associated crypts” surrounding the mucosal lymphoid follicles, but in these crypts there are two distinct axes of migration. Cells on one wall of the crypt differentiate into absorptive enterocytes, goblet, and enteroendocrine cells that migrate onto the villi, whereas cells on the follicle-facing wall of the same crypt acquire features of M cells and distinct follicle-associated enterocytes (6, 24, 25, 49). These FAE features include a lack of polymeric immunoglobulin receptor expression (47), M cell-like glycosylation patterns (25), and expression of vimentin (24), as described above. As they emerge from the crypt, differentiating M cells begin endocytic activity, fail to assemble brush borders, and acquire immune cells in their characteristic intraepithelial pocket (6). These observations suggest that inductive factors from the follicle act early in the differentiation pathway, inducing crypt cells to commit to FAE phenotypes.

Mucosal lymphoid follicles appear to play an important role in the induction of FAE and M cells, but the factors that determine the location of follicle assembly are not known. Induction of new lymphoid follicles occurs during mucosal inflammation: an inflamed ileal mucosa may contain increased numbers of mucosal follicles and an increased number of FAE and M cells (11). Experimental induction of follicles by injection of Peyer’s patch lymphocytes into the circulation of severe combined immunodeficient (SCID) mice (49) or into the submucosa of syngeneic normal mice (30) resulted in local assembly of new lymphoid follicles and the de novo appearance of FAE with typical M cells. Several lines of evidence indicate that B cells play a crucial role in this phenomenon: in immunodeficient SCID mice, injection of fractions enriched in B cells was most effective in reconstituting mucosal follicles (49). Furthermore, in B cell-deficient mice, mucosal follicles and identifiable M
cells are rare or absent (N. Debard, S. Kernenis, G. Fischer, E. Pringault, and J. Kraehenbuhl, unpublished data), whereas T cell-deficient nude mice have small Peyer’s patches with FAE and M cells (14).

All of the data summarized above are consistent with the hypothesis that the FAE/M cell phenotypes are induced by the local influence of cells or factors from the follicle or undifferentiated cells in adjacent crypts. On the other hand, there is evidence that factors or cells from the follicle or the lumen may also act later, to convert FAE enterocyte-like cells to antigen-transporting M cells. This hypothesis is supported by several observations. First, cells with both enterocyte and M cell features are present in FAE (24). Second, M cell numbers can increase rapidly after Salmonella infection (50) and after challenge with a non-intestinal bacterium (37), in time scales too short to be explained by induction of M cells in crypts. Third, the ability of B cells to convert an enterocyte phenotype to an M cell phenotype was directly demonstrated using a novel coculture system (30). Murine Peyer’s patch lymphocytes, when applied to the basolateral side of Caco-2 cell monolayers, entered the epithelium to reside in intraepithelial pockets reminiscent of those under M cells in vivo. The presence of lymphoid cells in the epithelium induced the appearance of M cell features: disorganization of the Caco-2 cell brush borders, loss of epithelium induced the appearance of M cell features in the coculture system (30). This system provides a new opportunity to study the molecular basis for lymphocyte-M cell interactions and the mechanisms underlying induction of M cells both in the follicle-associated crypts and in the FAE.

MECHANISMS OF ADHERENCE AND TRANSPORT

The ability of M cells to conduct transport of intact macromolecules from one side of the barrier to the other involves the directed movement of membrane vesicles. Although the molecular mechanisms of this transport have not been studied in M cells, it is safe to assume that the vectorial membrane traffic conducted by M cells depends on the polarized organization and signaling networks typical of polarized epithelial cells (12), and the relevance of these mechanisms to M cells have been reviewed (38). M cells are unique among epithelial cells in that transepithelial vesicular transport is the major pathway for endocytosed materials. Ultrastructural studies have shown that endocytic vesicles formed at the apical surface of M cells first deliver their cargo to endosomes in the apical cytoplasm (6, 40, 44, 54) and that these acidify their content and contain proteases (1, 21). Whether this invasicular milieu alters the antigens delivered into the pocket is not known.

Adherent particles, microbes, or macromolecules are effectively concentrated by adherence and may be transcytosed up to two orders of magnitude more efficiently than nonadherent materials (40). Because adherent antigens tend to elicit strong secretory (and often systemic) immune responses and these responses appear to be initiated in sites such as Peyer’s patches (7, 53), M cell adherence is thought to be a key event in induction of mucosal immunity. M cells also endocytose and transport solutes in the fluid content of endocytic vesicles (40, 44), and it is possible that transport of very small aliquots of soluble antigens over time may play a role in immune tolerance to soluble food antigens. The exact relationship of M cell transport activity to either mucosal or systemic tolerance, however, is not clear.

The ability of M cells to bind and transport adherent macromolecules, particles, and microorganisms was recognized by early investigators, but the mechanisms involved are still unclear. For example, it is not known why hydrophobic particles and cationic macromolecules such as polystyrene or latex beads (46), polylactide/polyglycolide microparticles (13), liposomes (8, 55), monoclonal IgA, monoclonal IgA-antigen complexes, and polyclonal secretory IgA adhered to apical membranes of M cells. This has been observed in suckling rabbits as a local accumulation of milk secretory IgA on M cells of Peyer’s patches (48) and in adult rabbits and mice, in which monoclonal IgA, monoclonal IgA-antigen complexes, and polyclonal secretory IgA adhered to apical membranes of M cells and were transported into the intraepithelial pocket (54).

INTERACTION OF SECRETORY IgA WITH M CELLS

Secretory IgA is produced by lamina propria plasma cells and transported into the lumen by crypt epithelial cells throughout the gut. The FAE, however, does not participate in IgA secretion, since it does not express basolateral polymeric immunoglobulin receptors (47). This is consistent with evidence that mucosal lymphoid follicles contain precursors of IgA B cells but are not sites of terminal plasma cell differentiation or IgA production (7, 53). Once secreted into the lumen, IgA does not adhere to the apical surfaces of enterocytes but adheres selectively to the apical membranes of M cells. This has been observed in suckling rabbits as a local accumulation of milk secretory IgA on M cells of Peyer’s patches (48) and in adult rabbits and mice, in which monoclonal IgA, monoclonal IgA-antigen complexes, and polyclonal secretory IgA adhered to apical membranes of M cells and were transported into the intraepithelial pocket (54).

The purpose of the IgA-M cell interaction is an intriguing question. Does uptake of IgA-antigen complexes by M cells boost the secretory immune response or have some other modulating effect by directing antigens to mucosal cells that display IgA receptors? We have found evidence that in naive mice, IgA can promote uptake of antigens and induction of immune responses, using liposomes containing ferritin as a test antigen and coating them (or not coating them) with monoclonal IgA. When used for mucosal immunization via the rectum, the IgA coat enhanced the uptake of the liposomes and moderately enhanced the local rectal/colonic secretory immune response to ferritin over that seen with liposomes lacking the IgA coat (55). Furthermore, a novel set of experiments suggested that secretory IgA itself can serve as a mucosal vaccine carrier. Cotkowsky and colleagues (10) genetically engineered
a secretory component (SC) to contain a protective foreign epitope (a 10-amino acid linear epitope from the invasin of Shigella flexneri) and used SC-IgA complexes to deliver the epitope into mucosal lymphoid tissue. The “antigenized” SC was recognized by an invasin-specific monoclonal antibody, was able to bind dimeric IgA, and evoked immune responses that included antibodies against invasin when administered orally (10).

EXPLORATION OF THE M CELL PATHWAY BY PATHOGENS

Epithelial M cells of the intestine are continuously exposed to the lumen of the gut and are relatively accessible to attachment and invasion of pathogens. M cell adherence and transport of pathogens do result in the initiation of mucosal and/or systemic infections. At mucosal sites containing M cells the risk of local invasion is high, but the occurrence of mucosal disease may be reduced by the close interactions of the FAE with antigen-processing and antigen-presenting cells, and by the organization of mucosal lymphoid tissues immediately under the epithelium. The exploitation of M cell transport by specific viral and bacterial pathogens and the use of such microorganisms in attenuated vaccine delivery have been reviewed (36, 51). The interaction of enteric bacteria with M cells likely involves initial recognition (perhaps via a lectin-carbohydrate interaction) followed by more intimate associations that require expression of additional bacterial genes, processing of M cell surface molecules, activation of intracellular signaling pathways, and recruitment or reorganization of membrane and cytoskeletal M cell proteins. Viruses are unable to alter the M cell surface but can themselves be processed by proteases in the lumen to an M cell-adherent form (4, 42).

The apparent predilection of multiple viral and bacterial pathogens for M cells is not yet understood. Perhaps the distinctive surface glycoconjugates or other receptors on M cells are recognized by viral and bacterial adhesins. Alternatively, key membrane components are simply more accessible on M cells because of the lack of a rigid brush border and thick glycocalyx. In addition, the particle nature of microorganisms may impart M cell specificity. Evidence for this has emerged from studies in this laboratory designed to evaluate the accessibility of glycolipid receptors in intestinal epithelial cell membranes to particulate antigens (22). We used as a probe cholera toxin B subunit (CTB), which binds to the glycolipid ganglioside GM1 on diverse cell types. Whereas soluble FITC-labeled CTB (hydrated diameter 6.4 nm) bound to apical plasma membranes of all cell types in the rabbit small intestinal epithelium, colloidal gold-labeled CTB (diameter 28 nm) adhered exclusively to Peyer’s patch M cells. Thus association of ligand with particles can result in M cell-specific adherence. However, 1-μm CTB-latex particles failed to adhere specifically to any epithelial cell surface, showing that the apical surface glycoconjugates on M cells were sufficient to prevent access of bacteria-sized particles to the membrane bilayer (22). Bacteria can circumvent the glycocalyx barrier by producing surface extensions such as pili, secreting proteins that penetrate or enzymatically cleave components of the glycocalyx, and reorganizing host cell surfaces (20). Nevertheless, these studies indicated that the presence of an appropriate receptor is necessary but not sufficient for microbial attachment; the receptor must also be accessible to microbial ligands or adhesins.

The highly developed apical microarchitecture of enterocytes and the relative accessibility of M cell apical membranes in vivo must be considered when interpreting pathogenicity studies conducted in vitro. For example, studies using HT-29 and other enterocyte-like cell lines have identified galactosylceramide as an epithelial cell component that can serve as a receptor for binding of human immunodeficiency virus (HIV), and it has been proposed that this glycolipid could serve as an HIV receptor on human rectal epithelial cells (18). However, the human rectal enterocyte glycocalyx is comparable in thickness to that of enterocytes of other regions and species (34). We previously observed that HIV failed to penetrate the glycocalyx of rabbit or mouse enterocytes on villi or the FAE, but the virus did adhere to rabbit and mouse M cells and was transcytosed (3). Further studies are needed to establish whether the relevant glycolipid is present on human rectal M cells and whether HIV actually enters via this route. Nevertheless, it is possible that receptor accessibility could account for M cell-selective uptake that would deliver the virus directly to its target cells in mucosal lymphoid tissues.

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