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*EDITORIAL*

# Intestinal M cells: The fallible sentinels?

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## Abstract

The gastrointestinal tract represents the largest mucosal membrane surface in the human body. The immune system in the gut is the first line of host defense against mucosal microbial pathogens and it plays a crucial role in maintaining mucosal homeostasis. Membranous or microfold cells, commonly referred to as microfold cells, are specialized epithelial cells of the gut-associated lymphoid tissues (GALT) and they play a sentinel role for the intestinal immune system by delivering luminal antigens through the follicle-associated epithelium to the underlying immune cells. M cells sample and uptake antigens at their apical membrane, encase them in vesicles to transport them to the basolateral membrane of M cells, and from there deliver antigens to the nearby lymphocytes. On the flip side, some intestinal pathogens exploit M cells as their portal of entry to invade the host and cause infections. In this article, we briefly review our current knowledge on the morphology, development, and function of M cells, with an emphasis on their dual role in the pathogenesis of gut infection and in the development of host mucosal immunity.



**Key words:** M cell; Gastrointestinal; Development; Pathogenesis; Mucosal immunity

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## INTRODUCTION

The gastrointestinal (GI) tract, in addition to its role as an organ for nutrient absorption, represents a key interface between the host and its external environment. Since the GI tract has the highest recorded bacterial cell density of any microbial ecosystem[1], it is not surprising that the GI immune system is both extensive and complex. The GI tract contains more antibody-producing cells than in the spleen and lymph nodes combined, and it contributes the majority of the body's immunoglobulin production in the form of IgA secreted into the intestinal lumen[2,3]. The GI mucosa, due to its large surface area (200 times greater than the skin)[4], requires consistent monitoring for potentially harmful agents (such as pathogens) while discriminating these from harmless food and non-pathogenic antigens. Gut-associated lymphoid tissue (GALT), consisting of Peyer's patches (PP), appendix, and other lymphoid aggregates in the large intestine, plays crucial roles in the maintenance of homeostasis in the GI system. The membranous or microfold cell (M cell) in the Peyer's patches is one of the primary cell types responsible for the capability of the intestinal immune system to mount both immunological and mucosal tolerogenic responses to foreign antigens.

This review will briefly summarize the current knowledge on intestinal M cells, with the emphasis of its potential role in GI infection and immunity. However, it is worth noting that M cells are also present in other mucosa-associated lymphoid tissues (MALT), such as the bronchus-associated lymphoid tissue (BALT) and nasal-associated lymphoid tissue (NALT)[5].

## MORPHOLOGY AND FUNCTIONS OF THE M CELL

M cells are specialized epithelial cells forming part of the follicle-associated epithelium (FAE) which overlies the PP and other lymphoid aggregates. The most striking feature of the human M cell under light or electron microscopy is the absence of surface microvilli which are characteristic of the intestinal epithelial cells. Instead, the apical membrane of the M cell has a microfold (or membranous) topography (Figure 1)[6-8], and hence the name M cell. Like other epithelial cells, M cells form tight junctions to maintain a barrier function, albeit with different structural features and adhesion protein expression[9]. The basolateral membrane of M cells is invaginated, and forms many "pockets", which harbor infiltrating lymphocytes[10]. The formation of these "pockets" greatly reduces the intracellular distance that antigens have to travel and allows M cells to rapidly transport (within 10 to 15 min) antigenic materials to the basolateral membrane[11,12].

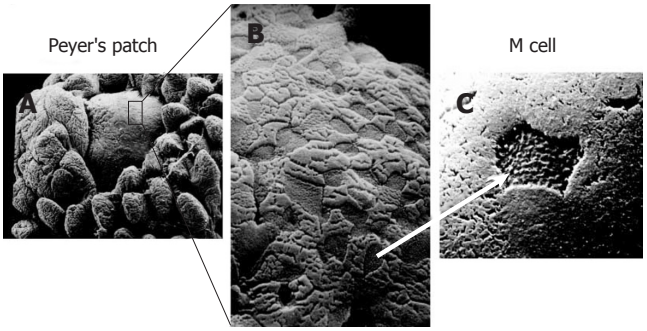


**Figure 1** Ultrastructure of the Peyer's patches and FAE (Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology[32], copyright 2003). **A**: At low magnification, the dome shape of the Peyer's patch protrudes between villi into the lumen of the intestine; **B**: At higher magnification, M cells can be seen as epithelial cells with surface microfolds rather than the microvilli that are seen on the surrounding conventional enterocytes; **C**: Antigen is taken up preferentially through M cells.

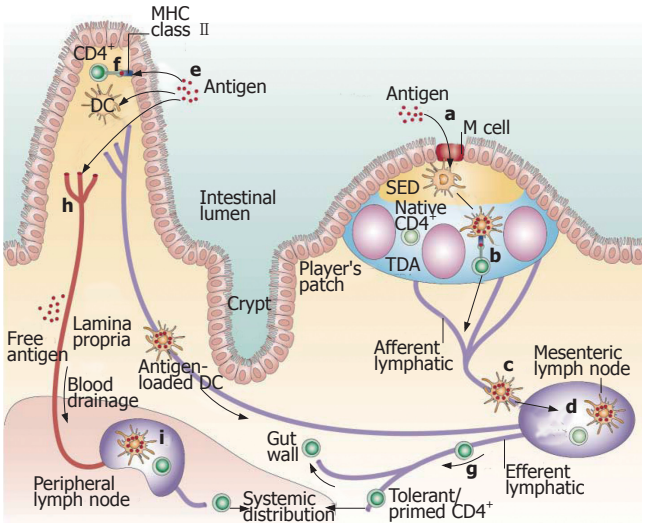


**Figure 2** Antigen uptake and recognition by $CD4^+$ T cells in the intestine. Antigen may enter through the microfold (M) cells in the follicle-associated epithelium (FAE) (**a**), and after transfer to local dendritic cells (DCs), might then be presented directly to T cells in the Peyer's patch (**b**). Alternatively, antigen or antigen-loaded DCs from the Peyer's patch may gain access to draining lymph (**c**), with subsequent T-cell recognition in the mesenteric lymph nodes (MLNs) (**d**). A similar process of antigen or antigen-presenting cell (APC) dissemination to MLNs may occur if antigen enters through the epithelium covering the villus lamina propria (**e**), but in this case, there is the further possibility that MHC class $II^+$ enterocytes may act as local APCs (**f**). In all cases, the antigen-responsive $CD4^+$ T cells acquire expression of the $\alpha4\beta7$ integrin and the chemokine receptor CCR9, leave the MLN in the efferent lymph (**g**) and after entering the bloodstream through the thoracic duct, exit into the mucosa through vessels in the lamina propria. T cells which have recognized antigen first in the MLN may also disseminate from the bloodstream throughout the peripheral immune system. Antigen may also gain direct access to the bloodstream from the gut (**h**) and interact with T cells in peripheral lymphoid tissues (**i**). (Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology[32], copyright 2003).

The morphology of M cells varies greatly amongst different animal species, and within anatomic sites of a species. For instance, the microfold structure is present only in human M cells[7], and human M cells lack microvilli. In contrast, the microvilli are present on the surface of murine M cells, but these are short and irregular[13] in contrast to the microvilli on the M cells of rabbit caecal lymphoid patches which are longer than the neighboring enterocytes[14]. The M cells express a wide range of carbohydrate markers with diverse glycoconjugate profiles[15], which perhaps allows M cells to interact with a broad range of microbes[16,17]. For example, while Ulex europaeus agglutinin-1 (UEA-1), an α-L-fucose residue-specific lectin which selectively labels fucose, recognizes M cells and goblet cells overlying the mouse PP[18,19], it fails to react with M cells on the mouse caecum or colon[15,20]. Conversely, UEA-1 does not bind to M cells of rabbit PP but reacts with those in the caecal lymphoid patches[21]. As a result, studies of rabbit M cells have frequently used vimentin, instead of UEA-1, as histochemical markers[22-25]. On the other hand, human M cells are generally negative for specific lectin binding[26], but are positive for the sialyl Lewis A antigen[27]. M cells in rats, guinea pigs and cats share similar lectin-binding patterns to enterocytes, although the cytokeratins 8 and 18 are over-expressed in M cells of rats and pigs, respectively, compared to neighboring enterocytes[28,29]. Because of these variations and diversity in the morphology and lectin-binding patterns, multiple confirmatory characteristics are usually required for the positive identification and characterization of M cells. Although glycosylation patterns and lectin-binding properties remain commonly used identifiers of M cells due to their relative ease of analyses, electron microscopy currently remains the most definitive method for M cell identification[15,20,30,31].

One of the major functions of M cells is believed to be the uptake and transport of antigens from the gut lumen to the underlying mucosal immune system (Figure 2)[32]. The apical membrane of M cells is specialized for the uptake and transport of antigens, featuring a reduced glycocalyx[33], and a general lack of membrane hydrolytic enzymes[34]. Additionally, the dramatic reduction of lysosomes may allow M cells to transport microorganisms into the lymphoid follicles without altering their antigenic properties[35]. M cells have been shown to be able to transport proteins[36,37], bacteria[31,38,39], viruses[40] and non-infectious particles[41,42] from the apical membrane to the basolateral surface. Bacteria and large particle transport is accomplished by phagocytosis, accompanied by apical membrane ruffling and actin cytosketeton rearrangements[38,43]. Under electron microscopy observation, M cells appear to reach out and engulf these large particles. Viral, and small adherent particles are endocytosed in clathrin-coated vesicles[41], while non-adherent antigens undergo fluid phase pinocytosis[6,11]. However, the role of M cells in antigen processing and presentation *per se* remains unclear. Although there have been several reports of M cells expressing major histocompatibility complex (MHC) class Ⅱ molecules[44-46], these findings could not be confirmed by others[32,47,48]. However, M cells do express cathepsin E, which is typically expressed on antigen-presenting cells[49], and M cells can also produce the pro-inflammatory cytokine interleukin 1 (IL-1)[50]. In addition, M cells are the main producers of CC chemokine ligand (CCL) 9 and CCL20 in the FAE[51], and also produce CXC chemokine ligand (CXCL) 16[52].

## DEVELOPMENT OF M CELLS

During embryonic and postnatal development, each crypt in the intestine is a clonal unit[53,54] whose cells differentiate into multiple types as they migrate. Cells on the villous side of the crypt differentiate into absorptive enterocytes, goblet cells and enteroendocrine cells. The cells on the FAE side of the crypt acquire the phenotype of absorptive enterocytes, M cells and, rarely, goblet cells[12,55,56]. Within this framework, two hypotheses have been proposed for the development of M cells. The first hypothesis suggests that M cells originate from a distinct cell lineage following an independent differentiation program. Evidence supporting this hypothesis includes that M cell development in the PP is restricted to specialized dome-associated crypts, and that both M cell precursors and their developmental intermediates have been identified within these dome-associated crypts[34,57] with early commitment of M cells observed in the mid-crypt area of caecum, appendix and PP[58,59]. In addition, the arrangement of some M cells as radial strips on the FAE dome with a single, predominating glycosylation pattern also implies M cell commitment occurs in the dome-associated crypts[14,60,61]. However, even in this example, the M cell glycosylation pattern is heterogenous.

The second hypothesis postulates that M cells develop from FAE enterocytes either as a developmental/transient stage of enterocytes, or in response to local signal stimulations (such as contact with lymphocytes and chemokines/cytokines). Indeed, Caco-2 cells, a human intestinal adenocarcinoma cell line, differentiate into an M cell-like morphology and phenotype after *in vitro* co-culture with murine PP-derived lymphocytes[39] or human B lymphoma cell lines. Moreover, intravenous injection of PP lymphocytes or normal bone marrow transplantation into severe combined immunodeficient (SCID) mice correlates with the development of M cells in the FAE[62,63]. The hypothesis that M cells are derived from enterocytes[64] is also supported by ultrastructural studies of chicken caecal tonsils[65], and by cell division and apoptosis studies in mouse PP[66] and in rabbit ileal PP[61]. Furthermore, a possible intermediate M cell/enterocyte cell type has been recently identified in upper regions of the dome in pigs[59].

Kerneis and Pringault have merged these diverse observations together into a single postulation that intestinal cell differentiation is largely determined by the crypt stem cells[67]. However, with the proper stimuli, alternate differentiation pathways could be followed (which they term "intestinal cell plasticity")[67]. In the case of M cells, enterocytes (perhaps immature) may convert into an M cell phenotype[67].

Although it is generally recognized that the mucosal lymphoid cells induce the development of the overlying specialized FAE, and that cell-to-cell contact and/or soluble factors provide important signals for the development of M cells[12], the events and signaling pathways directly involved in M cell differentiation and development remain poorly understood. The tumor necrosis factor (TNF) family of cytokines, particularly lymphotoxin (LT)-α, LT-β, and TNF-α produced by B cells, appear to play crucial roles in the development of Peyer's patches and FAE[68,69]. Their involvement in the development of M cells *per se* is, however, less clear. In the absence of LT-α and LT-β, the specialized areas of PP anlagen in the embryonic intestine are not formed[70-72]. Also, LT-β receptor-knockout mice lack PP[69]. However, mice whose B cells do not express LT-β do have normal FAE and M cells, although with smaller PP. Recombinase-activating gene (RAG)-1 -/- knockout mice, which lack mature B and T lymphocytes, have small PP-like aggregates having a normal M cell density[73]. When the LT-β receptor signaling is blocked by the antagonist lymphotoxin-β receptor-immunoglobulin G fusion protein in RAG-/- mice, the percentage of M cells in the PP-like aggregates decreases, suggesting that the LT-β signaling is essential for the differentiation and development of M cells, but LT-β signaling molecules could be supplied by other cell types in the absence of mature B and T lymphocytes[73]. On the other hand, mice having defective CD40 or IL-4 signaling, defective B-cell proliferation, or deficient in signal transducer and activator of transcription 6 (STAT6) have normal FAE and M cells[18,74]. Furthermore, although Toll-like receptors (TLRs) are expressed by the M cells[75-78], and exposure to bacteria can trigger TLR signaling resulting in induction of M cell proliferation[79] and up-regulation of transcytosis[80], TLR signaling does not appear to be essential for the development of M cells since MyD88-knockout, TLR-2 or TLR-4-knockout mice have normal M cell populations[18,79,81].

The notch signaling system is a recently characterized, highly conserved mechanism which regulates the differentiation, proliferation and apoptotic events at all stages of cell development, including the differentiation and renewal of intestinal epithelial cells and other types of intestinal cells, such as goblet cells, enteroendocrine cells, and Paneth cells[18,82,83]. Therefore, notch and notch ligands may play an important role in M cell developmental signaling. Indeed, the expression of Jagged-1 mRNA, a notch ligand, is increased in the *in vitro* M cell system compared to the parental epithelial cell line[84]. A subset of cells of the FAE in mice with a mutated Delta-3 gene, a notch ligand, showed abnormal apical membranes (dubbed as 'C cells'), and it has been suggested that these cells are precursor M cells[18]. This 'C cell' morphology has also been observed in normal mice[85].

## DISTRIBUTION OF M CELLS IN THE GI TRACT

In the human GI system, M cells are mainly found on the FAE overlying the dome structure of Peyer's patches in the small intestine[85,86]. The FAE, aside from having M cells, is distinguished by a reduced number of goblet cells and enterocytes[87,88]. Beneath the FAE lies the sub-epithelial dome (SED), a diffuse region of dendritic cells (DCs), naive B cells, $CD4^+$ and $CD8^+$ T cells, and macrophages[55]. Particles transported by the M cells from the lumen can be captured in SED by immature DCs[89], which then migrate to B-cell follicles and parafollicular T-cell zones and become mature DCs[90].

However, M cells are also present over lymphoid

follicles in the colon and rectum[91]. These follicles in the colonic crypts have a specialized epithelium with a greater proportion of goblet cells than PP, but fewer than the surrounding colonic regions. Similar to PP M cells, colonic M cells have transport vesicles, a thin glycocalyx, and a basolateral invagination containing pockets of lymphocytes[13].

The percentage of M cells comprising FAE varies substantially among host species and their anatomical locations, ranging from 5% to 10% in the human and murine PP[92] to about 50% in the rabbit and human caecal lymphoid aggregates[27,93].

## M CELLS AS SENTINELS OF THE GI IMMUNE SYSTEM

### *M cells and GI microbial infections*

The accessibility of M cells on the mucosal surface and their ability to transcytose particulate material make the M cells an ideal entry point for potential pathogens. Indeed, it has been demonstrated that M cells can transport a diverse array of mucosal microorganisms across the intestinal epithelial barrier, including bacteria (*Vibrio cholerae*[94], *Campylobacter jejuni*[95], *Mycobacterium tuberculosis*[13], *Shigella spp.*[96,97], *Salmonella spp.*[98,99], *Escherichia coli*[100,101], *Yersinia spp.*[102]), viruses (MMTV virus[74,103], polioviruses[104], reoviruses[105-107], prions[108] and HIV[40,109]) and parasites (Cryptosporidium[110]). In fact, many pathogens exploit the M cells as a conduit to invade the host and establish an infection. In this regard, enteropathogens, such as *Salmonella typhimurium*, *S. typhi*, *Shigella sp.* and *Yersinia spp.,* are capable of directly invading and destroying M cells and spreading the infection to neighboring enterocytes. For example, *S. typhimurium* initially invades the M cells[98,99], and induces a spotty and diffuse infection pattern with small groups of infected M cells[111]. Experimental infection in calves have shown that *S. typhimurium* is ingested by M cells within 5 min of contact[99,112]. The process ends with the exfoliation of majority of the infected M cells within 30 min, and cell death within an hour. This disruption of M cells allows the pathogen access to the neighboring enterocytes, and results in the sloughing off of the FAE[113,114]. Although these results have not been directly confirmed in humans, ulcerations are nevertheless present in regions corresponding to PP in cases of typhoid infection[115]. Similarly, free HIV particles use both, M cells and DCs, as conduits to infect local $CD4^+$ T cells[40,109,116]. In addition, it has been shown that the success of host adaptation of Salmonella in pigs is closely associated with the increased number of pathogens per M cell, as compared to the parental strain[117].

Other enteropathogens, such as Shigella species, are capable of attaching and adhering to M cells, but do not necessarily induce any cytotoxicity to the infected cells[97,118,119]. Instead, it induces membrane ruffling[96], and the afflicted M cells increase in size, rather than proliferating, to accommodate increased numbers of mononuclear cells in their basolateral pocket[96].

The interaction between intestinal pathogens and M cells are likely influenced and controlled by factors deriving from both the pathogen and the host. In this regard, the long polar fimbria (LPF) produced by the lpf operon and Salmonella pathogenicity island-1 (SPI-1) encoding the type Ⅲ secretory system play important roles in selective adherence of Salmonella to M cells[120,121]. LpfC or SPI-1 mutants of Salmonella show reduced colonization, decreased virulence, are not cytotoxic to M cells and are not disruptive to the FAE[99,122]. Transformation of the lpf operon into non-piliated *E coli* increased their uptake in PP[120]. Similarly, the uptake of Yersinia and Shigella by M cells is mediated by invasin or mechanisms encoded by a 30-kb virulence plasmid, respectively[102]. The presence or absence of these M cell-targeting gene products in pathogens might explain the differences seen amongst different strains of the pathogen in their attachment to M cells. For example, the rabbit diarrheagenic *E. coli* (RDEC)-1 strain is selective for adherence to M cells[100,101], whereas enterohemorrhagic *E. coli* (EHEC), such as strain O157: H7, has been found to attach to the FAE of human PP[123]. On the other hand, enteropathogenic *E. coli* (EPEC) is not transcytosed by the M cells and remains in the gut lumen.

It appears that most of the bacterial genes and their products identified to date for their invasive role represent the primary, but not the exclusive, mechanism for the entry of pathogens in M cells[124]. In Salmonella cases, some Salmonella serotypes, which are M cell selecting, lack the lpf operon, and others with the lpf operon do not target M cells[120,125]. Similarly, invasin-deficient Y. pseudotuberculosis mutants have delayed uptake of 3 to 5 d *in vivo,* but are nonetheless found in the spleen and liver at the same time and produce the same LD50 values, as the wild-type strain[126]. Perhaps, M cells can also recognize other Yersinia adhesins, such as pH 6 antigen and the plasmid encoded YadA, but with less affinity than for invasin[127]. In addition, the expression of the lpf operon has been found to cycle between 'off' and 'on', being referred to as phase variation[128,129]. It is probably an adaptation to avoid host defence. In this regard, cultivation in Lauria Bertani (LB) broth appears to increase the proportion of *S. typhimurium* in the lpf operon 'on'phase[128].

It is now recognized that the entry of M cells by intestinal pathogens is also mediated by a number of surface adhesion molecules, particularly those within the integrin family, of the host cells. In this regard, enteropathogenic Y. pseudotuberculosis can attach and invade murine M cells *via* β1-integrins expressed by the apical M cell membranes[130-132]. Studies have postulated that β1-integrins were the receptor for Yersinia invasin protein[131]. *In vitro* studies have demonstrated that α2β1 integrins are the exclusive heterodimer form found on the M cell apical membrane[133], although others have found that this heterodimer does not normally interact with invasin[134]. Other studies have shown that inhibition of α5β1 integrin expression on the apical membranes of Caco-2 cells and M cells *in vitro* abolished the abilities of these cells to transport microbes[77,135]. In addition, lymphotropic (X4) HIV transport by M cells is CXC4 receptor-mediated and is lactosyl cerebroside-dependent *in vitro*[109]. Finally, the variation of M cell glycocalyx has led to speculation about its role in pathogen tissue tropism[19,136].

### *Target M cells for mucosal immunization*

Just as pathogens can exploit M cells as the portal of entry for infection, biomedical researchers have, for many years, investigated the potential of using M cell-specific mechanisms for drug or vaccine delivery to the mucosal immune system[137-140]. Compared to parenteral routes, mucosal administration of drugs and vaccines is relatively simple, safe and inexpensive[141]. An additional benefit of mucosal immunization is its capability of priming and inducing both systemic and mucosal immune responses in the host[142,143]. Mucosal vaccination is necessary for protection against mucosal pathogens because parenteral immunization is generally ineffective for the development of protective mucosal immunity[144], and optimal vaccination strategies for many pathogens may require both mucosal and systemic delivery components[145].

Successful mucosal vaccines must circumvent the same barriers that mucosal pathogens have, i.e. mucus, proteases, nucleases, secreted antibodies, and the epithelial glycocalyx. Mucosal pathogens themselves have so far been the most effective models exploited for mucosal vaccination. The advantages of attenuated, live vaccines include their ability to activate multiple, innate immune responses. Currently, most effective oral vaccines are live attenuated poliovirus and live attenuated S. typhi, both of which exhibit selective binding to M cells and exploit M cell transport to the mucosal lymphoid tissue[99,104]. For this reason, other recombinant bacteria, including attenuated *Lactococcus spp.*, Listeria monocytogenes, and *Yersinia spp.*, have been constructed as delivery vectors for heterologous antigens[146-148].

M cells actively transport microparticles up to 1 μm in size, and those that are adherent to M cells are effectively transcytosed[12,33,149]. Thus, formulations that are multimeric or particulate and adhere to the mucosal surfaces, especially if there are some M cell specificities, seem most effective[145] while soluble, non-adherent antigens are frequently poorly internalized and hence generally induce weak immune responses or even immune tolerance[150]. The packaging of drugs and antigen microparticles on polystyrene or latex mircospheres protects them from degradation within the GI tract as well as allows them to be transcytosed by M cells[42,151-155]. Chitosan microparticles have shown promise both for oral vaccines and intranasal application[156-158]. Others have examined the potential of using copolymeric microparticles[159], proteosomes[160], liposomes[161], virus-like particles[162,163], and viral vectors, such as poliovirus and adenovirus[164,165]. However, these formulations can also bind to enterocytes[161], and are readily taken up by mucosal DCs. In addition, small vesicles derived from outer membrane components of bacteria[160,166] are interesting because of their uptake by M cells and DCs and their potential to induce an innate immune response through the activation of TLR pathways. Unfortunately, their tendency to become trapped in mucus necessitates large doses[165]. There are also regulatory concerns regarding the use of live, attenuated vectors for vaccine delivery, especially for use in immunocompromised population and the risk of reversion of the attenuated strain to full virulence.

Several recent studies have elegantly demonstrated the feasibility to specifically exploit M cells for mucosal vaccine development[167,168]. Manocha *et al*[167] have shown that HIV peptide bearing microparticles targeted to M cells, using UEA-1 lectins, are more immunogenic when administered mucosally than systemically. Wang *et al*[169] have used the adhesin protein sigma-1 from the enteric pathogen reovirus, which infects PP M cells, to direct DNA vaccines to the mucosal immune system[168]. Three expression plasmids encoding the genes for HIV gp160, cytoplasmic gp140, and secreted gp140 were conjugated to sigma-1 with poly-L-lysine and individually tested in mice. Intranasal immunization of mice showed specific, long-term CTL responses to gp160[168]. Upon challenge using a standard HIV surrogate test, these mice showed significant antiviral protection.

However, the relative importance of M cells in the induction of protective immunity by mucosal immunization remains unknown. For example, the antigen-specific immune responses as measured by IgG production is not substantially altered in the absence of PP[31]. Although M cells are capable of uptaking and transporting antigens, their role in antigen processing and presentation is less well characterized. In addition, M cells consist of only a small percentage of intestinal epithelial cells, raising the question of their overall efficiency in antigen uptake in the GI system. Moreover, there are redundancies at multiple levels of the mucosal immune system to ensure its continuing functionality. In this regard, intestinal DCs can migrate between mucosal epithelial cells, and directly sample the luminal antigens by forming transepithelial dendrites[170,171]. Other cell types, such as villous enterocytes, also express MHC class Ⅱ molecules and are capable of sampling and presenting intestinal antigens[172,173]. The difficulty in determining the precise role of M cells in the induction of mucosal immune responses is further confounded by the lack of availability of animal models which are completely and specifically deficient in M cells, making studies of intestinal antigen sampling by alternate cell types impossible.

## INTESTINAL VILLOUS M CELLS

Although M cells were initially believed to be exclusively located within the FAE region in the GI tract, this notion has been challenged by the recent identification and characterization of the intestinal villous M cells[31]. Intestinal villous M cells share all the known features of traditional M cells, but are independent of PP and not associated with the FAE. Instead, intestinal villous M cells lie on the intestinal villi either as small dense clusters (50 to 60 per animal) or diffusely. Intestinal villous M cells are more common in the terminal ileum than in other areas of the small intestine. Although the role and potential significance of these M cells remain to be elucidated, evidence to date indicates that they are functionally analogous to the PP M cells[31] and may compensate for PP M cell functions. Indeed, GALT-deficient mice produce antigen-specific IgG comparable with that produced in wild-type animals upon non-invasive bacterial challenge, and the population of UEA-1+ cells increased, perhaps the result of villous M

cells developing from epithelial cells upon exposure to foreign antigens or pathogens, such as *S. typhimurium*[31].

## PERSPECTIVES

More than three decades have passed since the first description of the M cell as the antigen shuttle for the mucosal immune system[6,7]. Current knowledge has highlighted the dynamic and complex role that M cells play in entry/invasion of pathogens, in antigen sampling, and in facilitating eliciting of immunity to GI infections. The advent of new technologies, such as confocal laser scanning microscopy and the intracellular visualization by use of fluorescence techniques, have supplemented the initial static electron microscopy studies in the characterization of M cells. The ability to cultivate M cells *in vitro* has complemented the *in vivo* models, and makes the molecular analysis of M cell functionality possible. The host-pathogen interactions have shown the varying strategies of the pathogen in exploiting M cells as conduits to initiate an infection, while at the same time evading or circumventing host immune surveillances. However, much work remains to be done to clarify the cellular and molecular mechanisms of the attachment to and the uptake of pathogens by M cells, and the interaction between the M cell and the pathogen, particularly the downstream events are evoked by the M cell antigen transport. Also, how does this transport lead to both mucosal and systemic immune responses? The presence of functional redundancies in the mucosal immune system and the lack of suitable animal models have further hindered the clarification of the precise role of M cells in the induction of mucosal immune responses and the rationale of targeting M cells for mucosal immunization. Further understanding and characterization of the mechanisms involved in the interaction between M cells and microorganisms, in the development and activation of M cells, and in the development of novel M cell targeting approaches will be needed for the development of a new generation of mucosal vaccines. In this regard, the recent identification of intestinal villous M cells and the rapid progress in our understanding of the role of TLR in the regulation of bacterial antigen uptake by M cells are likely to accelerate the development of M cell-based mucosal vaccines.

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