MINIREVIEW

M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis

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Abstract

M-cells are specialized cells found in the follicle-associated epithelium of intestinal Peyer's patches of gut-associated lymphoid tissue and in isolated lymphoid follicles, appendix and in mucosal-associated lymphoid tissue sites outside the gastrointestinal tract. In the gastrointestinal tract, M-cells play an important role in transport of antigen from the lumen of the small intestine to mucosal lymphoid tissues, where processing and initiation of immune responses occur. Thus, M-cells act as gateways to the mucosal immune system and this function has been exploited by many invading pathogens. Understanding the mechanism by which M-cells sample antigen will inform the design of oral vaccines with improved efficacy in priming mucosal and systemic immune responses. In this review, the origin and morphology of M-cells, and their role in mucosal immunity and pathogenesis of infections are discussed.

Introduction

The mucosal-associated lymphoid tissue (MALT), consisting of immunoreactive cells and organized lymphoid tissues, is found in close contact with all mucosa throughout the body. In the intestine, it is termed gut-associated lymphoid tissue (GALT), which consists of both isolated and aggregated lymphoid follicles (Neutra *et al.*, 2001). These are the sites where antigen recognition and mucosal immune responses are initiated. Aggregated lymphoid follicles are found in Peyer's patches (PP) of the small intestine, appendix vermiformis, and caecum, colon and rectum patches. GALT is one of the largest lymphoid organs in the body, containing up to 70% of the body's immunocytes. Typical GALT structures can be seen in PP, aggregated lymphoid follicles in the small intestinal mucosa. PP are named after the 17th-century Swiss anatomist Hans Conrad Peyer and are located along the antimesenteric side of the small intestine. Morphologically, PP are separated into three main domains: the follicular area, the parafollicular area and the follicle-associated epithelium (Neutra *et al.*, 2001). The follicular and parafollicular areas consist of the PP lymphoid follicle, which has a germinal centre containing proliferating B-lymphocytes, follicular dendritic cells (FDC) and macrophages. The follicle is surrounded by the corona, containing small lymphocytes expressing IgM and IgD; a dome lies above the follicle and contains B- and T-lymphocytes, dendritic cells (DCs) and macrophages. The follicle-associated epithelium (FAE) is a one-cell-thick layer composed of enterocytes and specialized epithelial cells termed M-cells (Owen & Jones, 1974). The FAE overlies the PP and forms the interface between the intestinal lymphoid system and the intestinal luminal environment. The function of PP was unknown until 1922, when Kenzaburo Kumagai identified uptake of *Mycobacterium tuberculosis* at the dome epithelium. However, he also observed uptake of heat-killed bacteria, sheep red blood cells and India ink by PP and hence concluded that this uptake was a nonspecific process. With the development of techniques for ultrastructural analysis in 1972, M-cells capable of taking up antigen were identified and the role of PP in the immune system became clear (Owen & Jones, 1974).

M-cells

M-cells are specialized epithelial cells found in the FAE of PP, and in isolated lymphoid follicles, appendix, and in

MALT sites outside the gastrointestinal tract. M-cells differ morphologically and enzymatically from adjacent enterocytes. In 1965, M-cells were first identified in rabbit appendix by J.F. Schmedtje (Owen & Jones, 1974). They were initially called lymphoepithelial cells but were later renamed M-cells, when Owen & Jones (1974) used electron microscopy to study PP of human small intestine and found the presence of 'microfolds' on the apical surface of these epithelial cells. M-cells act as gatekeepers to the mucosal immune system, continuously sampling the lumen of the small intestine and transporting antigen to the underlying mucosal lymphoid tissue for processing and initiation of immune responses (Kraehenbuhl & Neutra, 1992; Neutra *et al.*, 1996a, b). This M-cell sampling process has been exploited as a means of translocating the epithelium by pathogens including *Salmonella typhimurium* (Jensen *et al.*, 1998).

Origin and development of M-cells

The origin of M-cells within the FAE remains unclear and is the subject of much debate. It is known that intestinal epithelial cells in the FAE originate from stem cells in crypts located between a villus and a PP dome. Each crypt harbours a ring of stem cells that generate distinct cell types and there are two distinct axes of migration and differentiation (Heath, 1996). Cells located on the villous side of the crypt differentiate into absorptive enterocytes, goblet cells and endocrine cells as they migrate upwards in columns along the villous (Sierro *et al.*, 2000). Cells enter programmed cell death as they reach the tip of the villous and are shed into the intestinal lumen (Sierro *et al.*, 2000). Cells on the FAE side of the crypt move into the dome and differentiate into absorptive enterocytes and M-cells (Garvrieli *et al.*, 1992).

While it is accepted that enterocytes and M-cells have a common precursor, it is not known whether crypt cells commit early to FAE and M-cell phenotypes, or whether factors act later to induce further differentiation of enterocytes into M-cells (Nicoletti, 2000). Certainly, an established *in vitro* coculture system demonstrates that human intestinal enterocytes are converted to M-like cells by murine Peyer's patch lymphocytes (PPL) (Kerneis *et al.*, 1997). In this *in vitro* model, PPL migrate into spaces between epithelial cells, forming intraepithelial pockets in enterocytes with disordered apical microvilli, characteristic features of an M-cell (Kerneis *et al.*, 1997). However, whether this reflects the mechanisms governing *in vivo* differentiation is unclear as the *in vitro* system utilizes adenocarcinoma cells (Caco-2) that do not behave like normal enterocytes. Furthermore, a recent study found that prior treatment of Caco-2 epithelial monolayers with a human lymphocyte cell line is not absolutely required for differentiation to the M-like cell phenotype (Blanco & DiRita, 2006a). The presence of M-like cells in untreated Caco-2 monolayers was confirmed by the observation of cells lacking an organized brushborder and the presence of the M-cell marker, sialyl-Lewis A antigen (Blanco & DiRita, 2006a).

Combining histochemical and ultrastructural techniques, Gebert *et al.* (1999) analysed PP dome epithelium development and found M-cell precursor cells in specialized dome-associated crypts. These crypts differ from ordinary crypts in size, shape, cellular composition and location. Thus, this study suggests that M-cell differentiation is restricted to specialized crypts and is not induced by lymphocytes. Furthermore, a study by Gebert *et al.* (1999) detected M-cell precursors in the early proliferative zone of the crypts using M-cell-specific monoclonal antibodies. This observation again supports the theory that M-cells arise from a distinct cell lineage.

M-cell morphology

M-cells are distinguished from intestinal enterocytes by distinctive morphological features. At the apical surface, M-cells display a poorly organized brush border with short irregular microvilli, in contrast to the highly organized brush border of enterocytes, with uniform densely packed microvilli (Figs 1 and 2) (Kerneis *et al.*, 1997). Immunostaining of the microvillar proteins, actin and villin, has been used to identify M-cells that are detected by the absence of staining due to their short, irregular microvilli (Kanaya *et al.*, 2007). The usually thick glycocalyx associated with absorptive cells is absent in M-cells and is replaced by a thin glycocalyx, which is thought to aid greater access to antigens in the gut lumen. M-cells lack certain enterocyte apical surface glycoproteins. These include alkaline phosphatase and sucrase-isomaltase activity, which are typical to the brushborder of enterocytes, and both have been used as negative markers for M-cells (Gebert *et al.*, 1996). A lack of

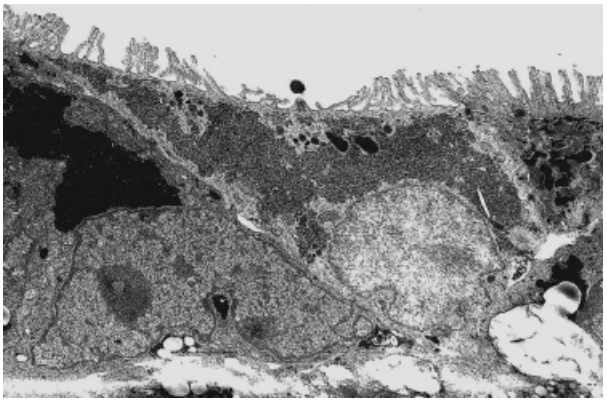

Fig. 1. Electronmicrograph of an M-cell from a Caco-2 coculture experiment displaying short irregular microvilli (Corr *et al.*, 2006).

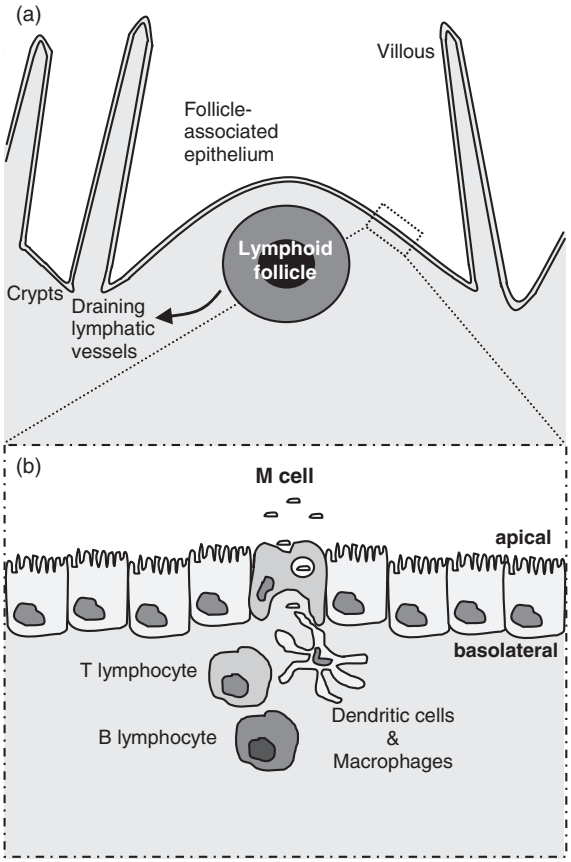

Fig. 2. Overview of M-cell location within the PP FAE.

alkaline phosphatase has been used to signify M-cells in mice, rabbits, rats, dogs and humans (Gebert *et al.*, 1996). At their basolateral surface, M-cells possess a unique intraepithelial invagination or 'pocket' (Neutra *et al.*, 1996a). The M-cell pocket contains B- and T-lymphocytes, macrophages and DCs. It provides a docking site for lymphocytes and other antigen-presenting cells, reducing the distance from the apical to the basolateral surface that transcytotic vesicles travel (Trier, 1991). The basolateral surface of epithelial cells contains two major subdomains: the lateral subdomain is involved in cell–cell adhesion and contains Na^+–K^+–ATPase, and the basal subdomain interacts with the extracellular matrix and the lamina propria (Trier, 1991).

While little is known about the glycoproteins or adhesion molecules expressed on the apical surface of M-cells, M-cells have been shown to display distinct glycosylation patterns as compared with their enterocyte neighbours (Gebert & Hach, 1993). Lectins have been used to characterize this glycosylation pattern. *Ulex europaeus* 1 (UEA-1) and *Psophocarpus tetragonolobus* (WBA) are two lectins that have been used to stain murine M-cells (Giannasca *et al.*, 1994). UEA-1 is specific for carbohydrate structures that contain α-1-fucose residues, and specifically stains the apical surfaces of M-cells (Giannasca *et al.*, 1999). These lectin-binding sites were also observed on M-cell plasma membranes, including the basolateral membrane, pocket domain and on intracellular vesicles (Neutra *et al.*, 1999). Furthermore, glycosylation patterns vary between M-cells within a single FAE, at different intestinal locations and between species (Gebert & Hach, 1993). Rabbit caecal M-cells selectively bind UEA-1 but UEA-1 is not bound by rabbit PP and M-cells in mouse caecal patches (Jepson *et al.*, 1996). Also, human colonic M-cells express intercellular adhesion molecule 1 (ICAM-1) but it is not expressed by PP M-cells (Jepson *et al.*, 1996).

Research is ongoing to determine the cellular markers of human M-cells. Significantly, recent work has utilized microarray analysis of Caco-2 cells differentiated to M-like cells and determined 180 differentially regulated genes. Of these potential targets, the molecule Galectin 9 was shown to be expressed on the apical surface of M-like cells and FAE and may provide a molecular marker of human M-cell differentiation (Pielage *et al.*, 2007). Monoclonal antibodies that are specific against single carbohydrate epitopes have also been used to identify M-cells in humans (Neutra *et al.*, 1999). Using this approach, M-cells have been shown to express sialyl Lewis antigen on their apical and subcellular membranes (Giannasca *et al.*, 1999).

M-cell function

The reduced brush border and lack of enzymatic activity of the M-cell apical surface suggests that they are unlikely to play a role in digestion or absorption. The exposed nature of the M-cell apical surface instead indicates that the primary function of M-cells is *trans*-epithelial transport. M-cells transport substances from the lumen of the intestine, across the epithelial barrier and to underlying immune cells where processing and initiation of immune responses occur. M-cells have been shown to transport particulates including latex beads, carbon particles and liposomes and macromolecules including ferritin, horseradish peroxidase, cholera toxin-binding subunit, lectins and antivirus antibodies (Gebert *et al.*, 1996). M-cells have also been shown to transport microorganisms including *Vibrio cholerae* and *S. typhimurium in vitro* (Kerneis *et al.*, 1997; Jensen *et al.*, 1998). This transport process occurs via *trans*-cellular endocytosis and has been shown to be temperature-dependent, transport being inhibited at 25 °C or lower (DesRieux *et al.*, 2005).

The *trans*-epithelial transport process occurs in three stages. Firstly, endocytosis of the substance occurs at the apical membrane, followed by transport of the substance via an endocytic vesicle to the endosomal compartment, and finally exocytosis at the basolateral membrane (DesRieux *et al.*, 2005). M-cell-mediated-translocation is very efficient and is a rapid process. As the M-cell cytoplasm above

the pocket is a thin apical rim, a complete endocytosis–exocytosis sequence can take a minimum of ten minutes (Kraehenbuhl & Neutra, 1992). The mechanisms by which M-cells take up microorganisms and molecules vary according to the nature of the material such as size, local surface pH, surface charge, hydrophobicity, concentration and the presence or absence of an M-cell-specific receptor (Des-Rieux *et al*., 2005). For example, large particles and bacteria can induce phagocytosis associated with apical membrane ruffling and rearrangement of the actin cytoskeleton (Liang *et al*., 2001). Viruses and other adherent particles are taken up by endocytosis via clathrin-coated vesicles (Owen, 1977). Nonadherent material is taken up by fluid-phase endocytosis and this has been shown in the case of soluble tracers such as ferritin (Neutra *et al*., 1987). A study by Neutra *et al*. (1987) found that a membrane-bound tracer, wheat germ agglutinin (WGA) lectin, is transported by M-cells *c*. 50 times more efficiently than the soluble tracer, bovine serum albumin (BSA).

During transcytosis, antigens do not undergo major ultrastructural alteration and are released intact into the pocket. While M-cells have a 16-fold decrease in lysosome volume and reduced lysozyme (acid phosphatase) activity compared with normal enterocytes, there is some evidence that M-cells may possess some enzymatic activity. Acid phosphatase-enriched prelysosomes, lysosome vesicles and major-histocompatability complex (MHC) class II determinants in the basolateral domain were detected in rat M-cells, suggesting a role in antigen processing and presentation (Allan *et al*., 1993). The aspartic proteinase Cathepsin E, normally found in the lysosomal compartment of antigen-presenting cells, has been localized in human and rat M-cells, again suggesting a role in processing and presentation (Finzi *et al*., 1993). Despite these observations, the extent of degradation and participation of M-cells in antigen processing and presentation remains unclear.

M-cells have other roles apart from antigen transport. It has been suggested that M-cells may aid immune response induction to the antigen they are transporting by releasing a costimulatory signal for T- and B-cell proliferation (Pappo & Mahlman, 1993). M-cells isolated from rabbit FAE were shown to secrete IL-1 following lipopolysaccharide stimulation *in vitro* and these culture supernatants were shown to induce T-cell proliferation (Pappo & Mahlman, 1993). This suggests that transcytosis of bacteria results in bacterial-lipopolysaccharide-induced IL-1 release, aiding proliferation of lymphocytes for induction of a mucosal immune response.

Entry sites for pathogens

M-cells are the main sites for continuous sampling and transport of antigens from the intestinal lumen to mucosal lymphoid tissues. Despite the production of antimicrobial peptides at the M-cell gateway (Goitsuka *et al*., 2007), this region of the FAE can be considered to be a potential 'Achilles heel' in the mucosal barrier and M-cells are exploited by many pathogens as a route of entry to underlying host tissues (Sansonetti & Phalipon, 1999). These include Poliovirus, *S. typhimurium*, *Yersinia enterocolitica* and *V. cholera* (Kerneis *et al*., 1997; Jensen *et al*., 1998; Ouzilou *et al*., 2002; Hamzaoui *et al*., 2004). As M-cells are capable of transporting inert particles, it has been suggested that pathogens may interact with M-cells via nonspecific passive mechanisms. However, several studies indicate that specific mechanisms of interaction mediate transport of microorganisms by M-cells (Tyrer *et al*., 2007). Understanding the mechanisms by which some microorganisms selectively use M-cells to cross the intestinal epithelium may aid development of disease control strategies. It may also allow exploitation of these invasion strategies for mucosal drug and vaccine delivery (Brayden *et al*., 2005).

Salmonella

Salmonella spp. are probably the most-studied pathogens that translocate M-cells. Murine ligated loop models have shown that M-cells are the major route of entry for *Salmonella* (Jones *et al*., 1994). *Salmonella typhimurium* exhibits selective targeting of M-cells overlying PP and can induce uptake, which is associated with extensive ruffling and FAE damage (Sansonetti & Phalipon, 1999). Adherence and invasion of M-cells induces apical membrane ruffling and actin polymerization, leading to bacterial engulfment, M-cell cytotoxicity and sloughing of the FAE (Jones *et al*., 1994). This FAE damage could potentially allow unrestricted bacterial invasion and may explain the occurrence of intestinal ulcers and perforations in typhoid patients (Jones *et al*., 1994). Rearrangements of FAE were exclusively observed at sites of M-cells in mice 30 min postinfection with *S. typhimurium*, with the integrity of enterocytes remaining intact. With increased length of exposure, increased M-cell membrane alterations were observed, with most M-cells displaying membrane protrusions and disruptions after 90 min. These disruptions result in pores in the epithelium that allow bacterial spread to organs before an immune response is established (Jones *et al*., 1994). The invasion machinery, encoded by genes located on the *Salmonella* pathogenicity island 1 (SPI1), is crucial for invasion of epithelial cells and also plays a role in M-cell invasion (Clark *et al*., 1998b). However, while mutants in SPI1 still invade M-cells they, are not cytotoxic and do not result in the destruction of the FAE (Jones *et al*., 1994). *Salmonella typhimurium* expresses a number of fimbrial operons, including *fim*, *lpf* and *pef*. These mediate binding to different murine epithelial cells and the *lpf* operon

appears to be responsible for specific adherence to murine M-cells (Baumler *et al.*, 1996). An *S. typhimurium* mutant in *lpfC* exhibited reduced colonization of PP and reduced destruction of M-cells, suggesting that *S. typhimurium* target M-cells via these fimbriae (Baumler *et al.*, 1996). Following transport across the FAE, *Salmonella* are phagocytosed by macrophages and DCs where they can survive within the phagocytic vacuole due to genes encoded by a second pathogenicity island (SPI2) (Baumler *et al.*, 1996).

Yersinia

Microscopic studies in mice have shown that *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* adhere to both enterocytes and M-cells, but demonstrate a preference for M-cells (Autenrieth & Firsching, 1996; Marra & Isberg, 1997; Sansonetti & Phalipon, 1999). Infection with *Y. enterocolitica* is accompanied by major damage to PP (Autenrieth & Firsching, 1996). It has been demonstrated that targeting of *Yersinia* to M-cells is mediated by invasin, an outer-membrane protein that binds to integrins of the β1 family expressed apically on M-cells (Autenrieth & Firsching, 1996; Marra & Isberg, 1997; Clark *et al.*, 1998a). Despite a recent study that showed that an invasin-deficient strain can still adhere to M-cells *in vitro*, *Yersinia* mutants lacking invasin protein display reduced colonization and translocation of PPs *in vivo* (Marra & Isberg, 1997; Hamzaoui *et al.*, 2004). Following M-cell uptake, bacteria must survive phagocytosis by macrophages. *Yersinia* have developed a mechanism of antiphagocytosis that allows them to remain extracellular (Forsberg *et al.*, 1994). This strategy is shared by the enteropathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*, and also the plague organism *Yersinia pestis*. Following adherence to the eukaryotic cell surface, the bacterium injects a set of Yop proteins (Yop-E, -H, -T, -O, -P and -M) into the cytoplasm via a Type III secretion system (Grosdent *et al.*, 2002). These Yop proteins induce breakdown of the F-actin network and Yop-O, also called YpkA, interrupts signalling pathways of phagocytosis (Forsberg *et al.*, 1994).

Shigella flexneri

Invasion of the intestinal epithelium by *Shigella* spp. causes the acute infection termed recto-colitis, better known as shigellosis. Inflammatory lesions occur on the upper rectum and distal colon. Upon further histopathological study, these ulcers were found to be located at sites of lymphoid follicles, suggesting that the FAE is a major route of entry (Sansonetti *et al.*, 1996). In rabbit ligated loop assays, *S. flexneri* enter M-cells after a short infection period (2–8 h), causing an increase in M-cell size due to accumulation of mononuclear cells in the pocket and thus acts as an increased area for transport (Perdomo *et al.*, 1994). This process is accompanied by extensive cellular damage and membrane ruffling; however, *S. flexneri* are not cytotoxic to M-cells (Perdomo *et al.*, 1994; Jensen *et al.*, 1998, Sansonetti & Phalipon, 1999). Following passage across the FAE, bacteria reinvade epithelial cells basolaterally and invasion is followed by an inflammatory process that disrupts the epithelium and increases permeability, facilitating further passage across the FAE (Perdomo *et al.*, 1994). Bacteria are then engulfed by macrophages and DCs. Two to four hours following infection, bacteria induce apoptosis by expressing four *ipa* genes encoding secretory proteins and an Mxi/Spa secretory apparatus. Bacteria release an IpaB invasin that binds to IL-1β-converting enzyme, causing cleavage of target proteins and cell death. Upon apoptosis, bacteria are released and spread from cell to cell, inducing the production of proinflammatory cytokines, IL-8 and TNF-α by enterocytes (Sansonetti & Phalipon, 1999; Phalipon & Sansonetti, 2007).

Vibrio cholerae

Binding of *V. cholerae* to M-cells occurs via a tight attachment domain that induces actin filaments to form structures that engulf the bacteria. Heat-killed bacteria do not attach to M-cells, indicating that the interaction requires specific bacterial adhesions (Blanco & DiRita, 2006a). A recent study found that *V. cholerae* is transcytosed by M-cells via interaction of cholera toxin and ganglioside receptor, GM_1 (Blanco & DiRita, 2006a, b). In this study, heat-killed bacteria were not transcytosed as heat-killing may destroy or remove cholera toxin required for binding to GM_1. Following transport across M-cells, *V. cholerae* infect lymphoblasts and macrophages, but this is accompanied by subsequent killing of the bacteria in the follicle dome (Davis & Owen, 1997). Uptake induces a host sIgA response to cholera toxin and the cholera lipopolysaccharide (Pierce *et al.*, 1987). However, pathogen-bound sIgA may then further enhance M-cell-mediated uptake of *V. cholerae* through targeting of M-cell sIgA receptors (Blanco & DiRita, 2006b).

Escherichia coli

Most strains of *Escherichia coli* do not adhere to M-cells, with the exception of some pathogenic strains. These include *E. coli* 0:124, enteroaggregative and diffuse adhering *E. coli*, enterotoxigenic *E. coli* and enteropathogenic *E. coli* rabbit diarrhoeagenic *E. coli* (RDEC)-1 (Cantey & Inman, 1981). The RDEC-1 strain of *E. coli* is an enteroadherent bacterium that adheres to the lymphoid follicle epithelium of ileal PP in postweanling rabbits (Inman & Cantey, 1983). In an ultrastructural study, *E. coli* RDEC-1 were seen to adhere specifically to M-cells of the lymphoid follicle epithelium; however, this did not result in their internalization (Cantey & Inman, 1981). Adherence is accompanied by blebbing and notching of the M-cell microvilli leading to the

eventual formation of vesicles. Adherence to absorptive epithelial cells causes pedestal formation by the plasmalemma that cup the bacteria (Cantey & Inman, 1981; Marchetti *et al.*, 2004). A recent study found that nonmotile *E. coli* RDEC-1 do not efficiently adhere to appendix M-cells of the rabbit (Marchetti *et al.*, 2004). The enterohaemorrhagic *E. coli* strain O157:H7 selectively adheres to human FAE PP but it is not known whether it specifically adheres to M-cells. However, the protein intimin (γ), essential for its colonization, binds β1-integrins, and these are expressed on the M-cell apical surface (McKee *et al.*, 1995; Hamzaoui *et al.*, 2004). A recent study observed similar rates of translocation of enteropathogenic *E. coli* O127:H7 across an *in vitro* M-cell model compared with normal enterocytes (Martinez-Argudo *et al.*, 2007). Furthermore, in this study, it was observed that translocation rates were significantly increased in the absence of a functioning TypeIII secretion system (Martinez-Argudo *et al.*, 2007).

Listeria

Considerable research work has centred on the precise route by which the food-borne pathogen *Listeria monocytogenes* gains access to Intraepithelial lymphoid cells and mucosal lymphoid tissues. It has been well documented that *L. monocytogenes* can invade nonphagocytic cells (using bacterial internalin proteins) and that this process is critical for bacterial translocation of the intestinal epithelium (Vázquez-Boland *et al.*, 2001; Hamon *et al.*, 2006). Pentecost *et al.* (2006) have recently demonstrated that the pathogen binds to E-cadherin exposed at the villous tips during extrusion of epithelial cells in this region. Previous work has demonstrated that this interaction is host specific and that human, but not murine and rat cells are efficiently targeted by *L. monocytogenes* (Lecuit *et al.*, 1999).

While it is clear that the pathogen invades host cells through a specific interaction between internalins and target receptors, some speculation surrounds whether the pathogen also has the potential to invade via M-cells for rapid translocation. This interaction would effectively prime the host immune response but could also be utilized by the pathogen as a means for accessing the underlying mucosa. Indeed, some evidence for M-cell sampling of *L. monocytogenes* has emerged from studies in mice and rats (Pron *et al.*, 1998; Daniels *et al.*, 2000). *In vivo* analysis of orogastric *L. monocytogenes* infections using animal models has demonstrated preferential replication within the PP with extremely rapid translocation of the bacterium (within 15 min) to internal organs (Pron *et al.*, 1998). Similarly, a more recent study using the murine model of infection (Daniels *et al.*, 2000) found very rapid translocation of *Listeria* from the lumen of the gastrointestinal tract to internal organs. Other studies also demonstrated rapid localization of *L. monocytogenes* within the PP of mice (Marco *et al.*, 1997; Corr *et al.*, 2006). It is evident that in the absence of a specific interaction between *L. monocytogenes* and murine host cells, the pathogen may be sampled within PP, providing for rapid translocation.

The *in vitro* model of M-cell development was recently utilized for analysis of *L. monocytogenes* translocation. This work demonstrates that the pathogen migrates through differentiated M-cells at rates similar to translocation of *V. cholerae* and more efficiently than control *Lactobacillus salivarius* cells. Furthermore, this interaction is independent of internalin expression or expression of the major virulence factor Listeriolysin (Corr *et al.*, 2006). Using a similar model, Daniels *et al.* (2000) indicated that while *L. monocytogenes* attaches to and invades both enterocytes and M-cells, no preferential targeting of M-cells occurs. However, they did not examine translocation of the monolayer.

While elegant work has unravelled the specific mechanisms by which *L. monocytogenes* directly invades host cells (Hamon *et al.*, 2006; Pentecost *et al.*, 2006), there is much evidence to support a role for M-cells in sampling the pathogen from the intestinal lumen leading to rapid translocation to internal organs (Pron *et al.*, 1998; Corr *et al.*, 2006).

Viruses

Several viruses are transported by M-cells and reovirus type-1, poliovirus and HIV type 1 (HIV-1) bind specifically (Sicinski *et al.*, 1990; Amerongen *et al.*, 1991, 1994). Reovirus, an orally transmitted murine pathogen, affects the nervous system, causing encephalitis. Reovirus type-1 selectively adheres to ileal and colorectal M-cells, and this is mediated by its outer capsid proteins (Amerongen *et al.*, 1994). Gastrointestinal transit causes proteolytic cleavage of the outer capsid proteins, which become activated for adherence (Helander *et al.*, 2003). The receptors for reovirus type-1 are α-2-3-linked sialic acid glycoconjugates that bind the viral haemaglutinin sigma 1 (Davis & Owen, 1997). Infection causes depletion of M-cells from the FAE, which may affect host antiviral responses; however, the virus is cleared within 10 days in mice by production of antiviral sIgA (Silvey *et al.*, 2001).

Poliovirus is the causative agent of poliomyelitis and infects humans by the oral route. It is thought that the primary sites of replication in the gut are PP (Sicinski *et al.*, 1990). In human tissues infected with poliovirus, virions were found to be specifically adhering to the surface of M-cells and in vesicles within M-cells (Sicinski *et al.*, 1990). Poliovirus type-1 and the corresponding attenuated vaccine strain, Sabin, both translocate across M-cells *in vitro* (Ouzilou *et al.*, 2002).

Transmission of HIV-1 infection via anorectal, cervicovaginal, foreskin and urethral epithelia accounts for 80% of

AIDS cases (Amerongen *et al.*, 1991). HIV-1 must cross the mucosal barrier of the intestinal or genital tracts to infect $CD4^+$ T-cells and translocation across epithelial cells or M-cells of FAE of intestinal and tonsil lymphoid follicles may play a role in infection (Amerongen *et al.*, 1991). Infection is thought to occur when epithelial barriers are damaged, although studies in monkeys with simian immunodeficiency virus (SIV) have shown that infection can occur via intact rectal mucosa (Amerongen *et al.*, 1991). HIV-1 has been shown to adhere to M-cells but not enterocytes on villi or in the FAE in rabbits and mice (Amerongen *et al.*, 1991). Recently, it was shown that HIV-1 is transported across M-cells via lactosyl cerebroside and the chemokine receptor, CXCR4 (Fotopoulos *et al.*, 2002). They demonstrated that a lymphotropic (X4, syncytium-inducing HIV-1) HIV-1 strain crosses M-cells via lactosyl cerebroside and CXCR4, receptors that are expressed apically on M-cells. A monotrophic (R5, nonsyncytium inducing) HIV-1 strain was unable to cross M-cells because they do not express its CCR5 receptor; however, transfection of M-cells with CCR5 cDNA restored translocation.

Prions

Prion disorders affect the mammalian brain by causing plaques and lesions. Prion diseases include scrapie in sheep, bovine spongiform encephalopathies (BSE or mad cow disease) and the human disease, Creutzfeldt–Jakob disease. These are all caused by an infectious misfolded prion (PrP^{Sc}) that converts normal cellular prions to an abnormal form (Heppner *et al.*, 2001). Transmission of BSE to humans via dietary exposure has become a concern since the appearance of a new variant of Creutzfeld–Jakob disease (Heppner *et al.*, 2001). Using an *in vitro* M-cell model, it was shown that Rocky mountain laboratory scrapie prions are transported by M-cells while no transcytosis was observed in enterocytes (Ghosh, 2002). Follicular DCs have been shown to be important for development of prion pathogenesis and subsequent infection of the neural system and this supports the role of M-cells in prion transport (Ghosh, 2002).

An *in vitro* M-cell model

The study of M-cells has been problematic due to the difficulties associated with isolating M-cells. M-cells have been difficult to identify and isolate due to their low numbers in the FAE, lack of an enriched preparation and phenotype variation between species. M-cells have been difficult to characterize biochemically and so little is known about their cell biology. In the past, studies have relied on *in vivo* or *in situ* methods such as isolated tissue loops or explant cultures. However, the development of an *in vitro* M-cell/FAE model has provided a reproducible approach in which phenotypic and physiological features of the FAE and M-cells overlying PP are maintained (Kerneis *et al.*, 1997; DesRieux *et al.*, 2007). This model facilitates the study of antigen transport across M-cells, and their interaction with lymphocytes, bacteria and vaccines (Kerneis *et al.*, 1997). This system uses the human adenocarcinomal enterocyte-like cell line, Caco-2, to mimic M-cell activity through differentiation of epithelial enterocytes to M-cells via coculture with PPL or a Raji human lymphocyte cell line. Briefly, the M-cell model involves a two-chamber transwell system in which epithelial enterocytes are grown on a polycarbonate porous membrane until differentiation to a villous-expressing cell type. Upon differentiation, freshly isolated PPL are introduced into the basolateral chamber, where they migrate into the monolayer (Fig. 3). This coculture induces the M-cell phenotype (Kerneis *et al.*, 1997). The efficiency of the system has been determined by its ability to mimic key M-cell properties. Translocation of fluorescein isothiocyanate (FITC)-labelled microspheres and live noninvasive *V. cholerae* from the apical to basolateral chamber of cocultures, are significantly increased in Caco-2 cells grown in the presence of PPL, indicative of the sampling process (Kerneis *et al.*, 1997). Particle transport is temperature-dependent in cocultures indicating that a transcytotic route is involved (Tyrer *et al.*, 2002). Apical expression of alkaline phosphatase is downregulated in cocultures and a redistribution of the actin-associated protein villin from the apical surface to the cytoplasm indicates a loss of an organized brushborder. $\alpha5\beta1$ Integrin, normally expressed on the lateral and basolateral surfaces of Caco-2 cells, is present on apical membranes of cocultures (Schulte *et al.*, 2000). The M-cell *in vitro* model has shown great potential in the study of the interaction of pathogens with the intestinal epithelium and also the determination of molecular mechanisms required for translocation across M-cells (Schulte *et al.*, 2000). It also allows a method for determination of the factors and mechanisms that influence M-cell development and the development of oral vaccine delivery.

The future of M-cell research: vaccine delivery

Oral vaccines will have to overcome many hurdles in order to be effective. The hurdles include (1) poor accessibility of mucosal DCs due to dilution or degradation of the antigen, peristalsis and the mucus barrier, and (2) oral tolerance, which downregulates cell-mediated and humoral immune responses (Nicoletti, 2000). Given the unique features of M-cells and their specialized ability to transcytose numerous microorganisms and particulates, targeted delivery of antigens to M-cells may provide a mechanism for the efficient presentation of antigens to initiate a mucosal immune response (Brayden & Baird, 2004; Brayden *et al.*, 2005). The adhesins required for attachment of pathogens to specific

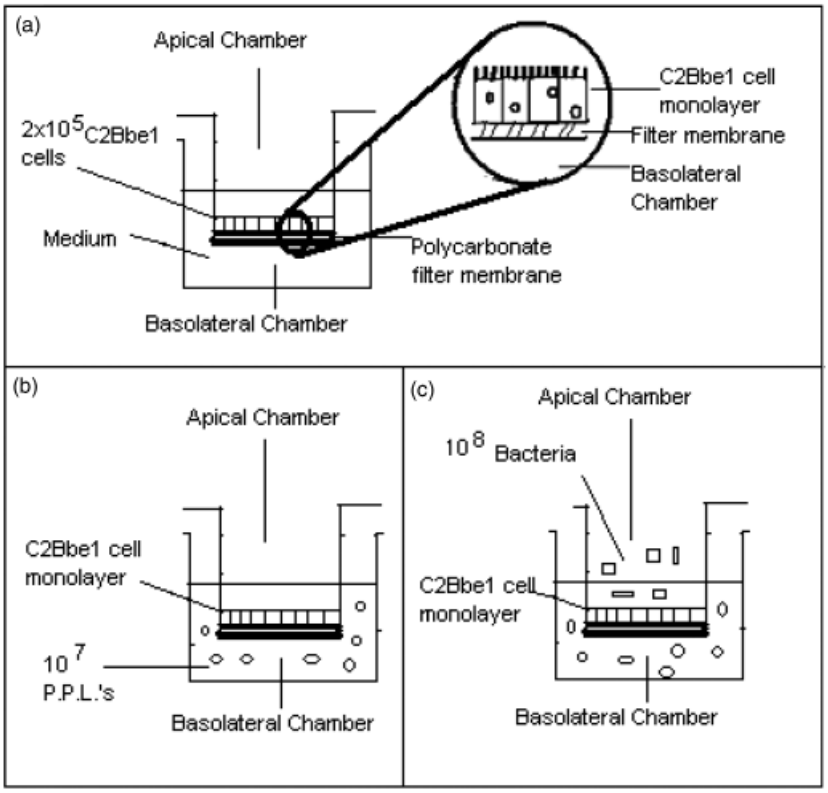

Fig. 3. Outline of the *in vitro* M-cell model (Corr *et al.*, 2006).

receptors on mucosal surfaces are being used for vaccine delivery via antigen-encapsulated microspheres (Wu *et al.*, 2001; Byrd *et al.*, 2005; Suzuki *et al.*, 2006). Rotavirus selectively binds to rabbit M-cells via the viral haemagglutinin adhesin, α-1 protein. Administration of rotavirus protein α-1 conjugated with polylysine produces antigen-specific serum IgG and mucosal IgA responses (Kim *et al.*, 2002). Biodegradable microparticles made from the copolymer poly-(DL-lactide-coglycide) (PLG) also show potential as mucosal delivery vehicles. PLG microspheres containing rotavirus antigen VP-6 DNA confer protection against challenge for up to 12 weeks. Microspheres coated with VP-6 are selectively taken up by sheep PP and induce VP-6-specific IgA following oral immunization of mice (Kim *et al.*, 2002). PLG microparticles containing HIV peptides in combination with *Ulex europaeus-1* lectin, which binds M-cell apical surfaces, generate both mucosal and systemic immune responses following intranasal immunization of mice (Manocha *et al.*, 2005). A recent study has demonstrated efficient uptake of PEGylated PLGA-based nanoparticles displaying surface RGD molecules that successfully targeted β1 integrins on human M cells in cell culture and murine M cells *in vivo* (Garinot *et al.*, 2007).

While particle uptake has been demonstrated *in vivo* in rodents and rabbits, it is unclear whether this is the case in humans. Human oral vaccine Phase 1 trials using *E. coli* enterotoxigenic *E. coli* (ETEC) antigens contained in biodegradable microspheres have been carried out but had poor outcomes and did not sufficiently stimulate mucosal immunity (Katz *et al.*, 2003). Several pathogens target M-cells as a mode of entry into the host and are being genetically engineered to deliver antigen to mucosal inductive sites for induction of antigen-specific immune responses (Suzuki *et al.*, 2006). Live-attenuated strains that have successfully been used as mucosal vaccines include the Sabin strain of poliovirus and *Salmonella typhi* Ty21a for polio and typhoid, respectively (Clark *et al.*, 2001a, b). Investigation into the use of *Salmonella* species as live vectors for delivery of antigens and DNA is underway and one mutant lacking DNA adenine methylase has been shown previously to be unable to induce M-cell cytotoxicity but has an enhanced ability to promote an immune response (Clark *et al.*, 2001a, b). Targeting ligand-coated particles to M-cells, combined with attempts to mimic pathogen entry routes via M-cells, could lead to increased uptake *in vivo* and successful initiation of mucosal immune responses.

Conclusion

Despite relatively few numbers of M-cells within the intestinal epithelium compared with normal intestinal enterocytes, the nature of M-cells provides them with an

important role in antigen sampling, bacterial translocation and initiation of mucosal immune responses. With the development of the *in vitro* M-cell model, research into these important immune cells has improved. However, the identification of specific M-cell antibodies and markers will significantly improve one's understanding of these cells. Furthermore, thorough research into the relationship between M-cells and the establishment of disease, and their ability to deliver antigen directly to mucosal immune initiation sites will improve delivery of existing mucosal vaccines and development of new strategies for oral vaccines.

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