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M-CELLS AND THEIR ROLE IN INFECTION: A REVIEW

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SUMMARY

M-cells are located in the epithelia overlying mucosal tissues where they function as the antigen sampling cells of the mucosal immune system. Paradoxically, M-cells are also exploited by a range of pathogens as a route of host invasion. Despite their pivotal roles in immunity and infection, the mechanisms responsible for micro-organism interaction with M-cells and the origin and developmental pathway of these specialised cells has, until recently, remained obscure. Now, the availability of improved methods for identifying M-cells coupled with a variety of modern research tools is permitting more detailed analysis of M-cell origin and function. The majority of evidence now suggests M-cells primarily arise from undifferentiated crypt cells under stimulation from the local lymphoid environment. However, under appropriate antigenic stimulation, enterocytes may also contribute to the M-cell population. The mechanisms by which pathogens target M-cells are now also partially defined and it has become clear that some of the microbial infection mechanisms previously defined *in vitro* similarly mediate M-cell infection. However, it also appears that M-cell invasion may proceed via additional routes not previously identified by *in vitro* studies. Furthermore, it is clear that M-cells, which constitute only a minute proportion of the gastro-intestinal epithelium, are not the sole site of microbial translocation from the gut. These findings indicate that many micro-organisms possess multiple alternate routes of intestinal epithelial invasion; reflecting the critical importance of this initial step in microbial infection. In this paper we review the current state of knowledge about the origin of M-cells and their role in pathogen invasion.

INTRODUCTION

Membranous epithelial (M-) cells transport antigens across mucosal epithelia to the underlying lymphoid tissues where protective immune responses are initiated (*Gebert* et al., 1996; *Neutra* et al., 1996; *Gebert*, 1997). Paradoxically, some micro-organisms exploit M-cells as a route of host invasion. The bulk of our knowledge of M-cells has been derived from studies of those present in the follicle-associated epithelium (FAE) overlying isolated and aggregated lymphoid follicles in the intestinal tract. The proportion of M-cells in the FAE

varies from 10% in humans and mice to 50% in rabbits and 100% in the terminal ileum of pigs and calves. The existence of cells analogous to M-cells has also been reported in the epithelia of a variety of other mucosal tissues in a diverse range of species. However, it should also be borne in mind that the presence of functional equivalents of M-cells at some of these sites has been disputed (for a critical review of the evidence for and against M-cell analogues at extra-intestinal sites see *Gebert* and *Pabst*, 1999).

In writing this review, it is not our intention to provide a thorough description of the morphology and function of M-cells or a comprehensive list of the pathogens suggested to utilise the M-cell route of infection; these topics have been covered extensively in other reviews (*Gebert* et al., 1996; *Neutra* et al., 1996; *Siebers* and *Finlay*, 1996; *Gebert*, 1997; *Owen*, 1998a; *Sansonetti* and *Phalipon*, 1999). Instead, we will focus primarily on recently published data that has advanced knowledge of M-cell function and development, and, most importantly, their role in infection. In considering these topics, we shall specifically concentrate on intestinal M-cells and their role in bacterial infection.

M-CELL CHARACTERISTICS AND THEIR IDENTIFICATION

The most important characteristic of M-cells is of course their ability to transport material across epithelial barriers (*Gebert* et al., 1996; *Jepson* et al., 1996; *Neutra* et al., 1996; *Gebert*, 1997). In addition to possessing an increased endocytic capacity for a broad range of materials, ultrastructural analysis has suggested that M-cells may have a reduced degradative capacity. In comparison to neighbouring enterocytes, intestinal M-cells generally have less organised brush borders which may promote interaction of material with the apical membrane. M-cells also have a characteristic basolateral invagination that harbours leukocytes. This provides a short-cut for transcytosis of antigens and permits their rapid delivery to lymphoid tissue where a mucosal immune response may be induced. These well-established morphological features of M-cells not only have implications to their function as antigen-sampling cells but also facilitate their identification by transmission electron microscopy (TEM). However, since TEM studies tend to focus on small numbers of cells they are not always the most appropriate means of examining a cell type such as M-cells, which constitute a relatively minor cell population. Furthermore, the interaction of bacteria and inert particles varies considerably both between and within individual Peyer's patches and regions of the FAE, necessitating the examination of numerous M-cells (*Clark* et al., 1994a, 1998a; *Frey* et al., 1996). The unusual brush border morphology of M-cells also facilitates their identification by scanning electron microscopy (SEM) which readily allows examination of almost the entire FAE and thus a greater proportion of the M-cell population. However, SEM only allows a view of the epithelial surface so is limited to studies of the binding of material to M-cells and any subsequent alterations in surface morphology. It yields little information about the uptake of material by M-cells and its subsequent fate. For these reasons, considerable effort has been put into developing methods of identifying M-cells at the light microscopical level. The most widely used cytochemical methods for M-cell identification rely on their relative lack of alkaline phosphatase (e.g.

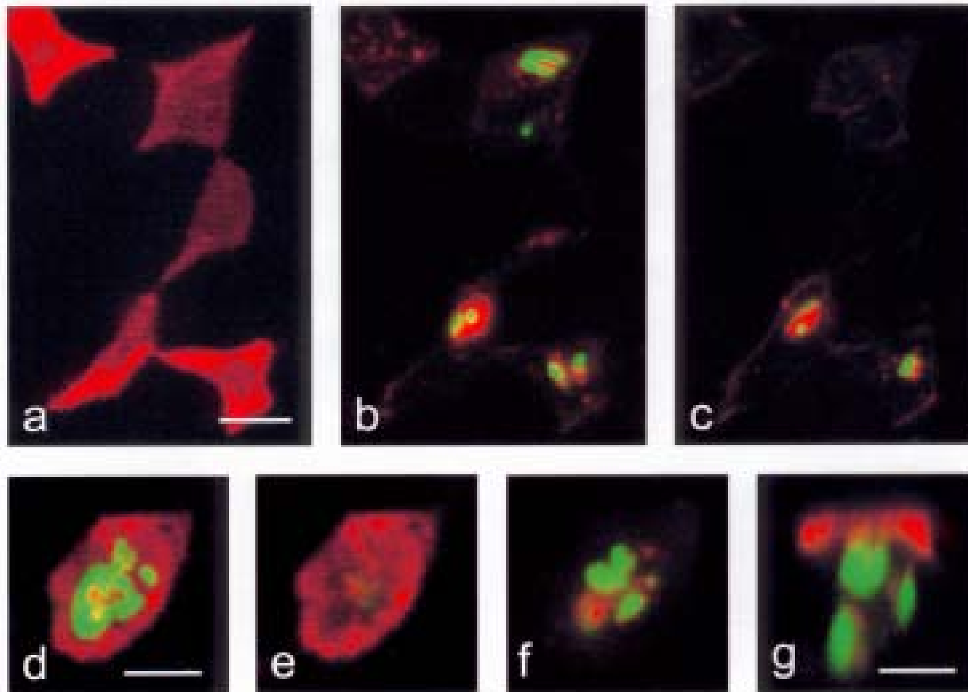

Figure 1: Confocal laser scanning microscope images of mouse Peyer's patch FAE from gut loops infected for 60 min with wild type *Y. pseudotuberculosis* strain YPIII/pIB1 constitutively expressing green fluorescent protein (GFP). The bacteria were grown in Luria Bertani (LB) broth at 26°C in a shaking incubator, and inoculated at a concentration of 2 x 10^9 CFU/ml directly into the gut loops. After tissue fixation, M-cells were localised with the lectin *Ulex europaeus* 1 (red).
(a)-(c): multiple bacteria are observed at 2 µm (b) and 4 µm (c) below the M-cell apical surfaces (a).
(d)-(g): a single M-cell is invaded by numerous bacteria. In (d), images from 0 to 8 µm depth have been projected to give a single image to show that very large numbers of bacteria are associated with this M-cell. Bacteria are absent from the M-cell surface (e), but multiple invaded bacteria are located at 3 µm below this level (f). Multiple invaded bacteria are similarly observed when this cell is viewed in vertical (x-z) section (g).
Scale bars = 5 µm.

Savidge et al., 1991; *Savidge*, 1996) or on their atypical expression of intermediate filament proteins (*Gebert* et al., 1992, 1994; *Jepson* et al., 1992) or glycoconjugates (*Clark* et al., 1993, 1994b, 2000; *Gebert* and *Hach*, 1993; *Jepson* et al., 1993a; *Giannasca* et al., 1994). Of these techniques, the positive cytochemical markers provided by antibodies to intermediate filaments and by lectins are more reliable and most used (e.g. Figure 1). However, there are some problems with the application of these markers which have been discussed extensively elsewhere (*Jepson* et al., 1996; *Jepson* and *Clark*, 1998; *Clark* et al., 2000). Specifically, each marker is only applicable to a limited range of species and/or sites due to great variation between different populations of M-cells (*Jepson* et al., 1993a, 1995a,b; *Clark* et al., 1994b; *Giannasca* et al., 1994). In addition, reliable positive markers for human M-cells are currently lacking. However, some lectins predominantly label human FAE al-

though without clear M-cell selectivity (*Jepson* et al., 1996; *Sharma* et al., 1996). Recent evidence has shown that the sialyl Lewis A antigen is preferentially expressed on a subset of human intestinal M-cells, although some expression on FAE enterocytes and goblet cells was also reported (*Giannasca* et al., 1999).

DEVELOPMENT AND REGULATION OF THE M-CELL POPULATION

There is controversy regarding whether M-cells can be formed from fully differentiated enterocytes within the follicle-associated epithelium (FAE) or whether their differentiation is pre-programmed in the crypts that supply epithelial cells to both the FAE and adjacent villi (*Gebert* and *Posselt*, 1997; *Gebert* et al., 1999; *Niedergang* and *Kraehenbuhl*, 2000). In either case, it can be assumed that local factors associated with the lymphoid tissue environment trigger M-cell development. There is now considerable evidence from studies of immunodeficient and knockout mice that B-lymphocytes are the predominant cell population involved in Peyer's patch development (*Savidge*, 1996; *Debard* et al., 1999; *Niedergang* and *Kraehenbuhl*, 2000). Although B-cells appear to promote M-cell development, the presence of small numbers of M-cells in B-cell knockout mice suggests that other cells may substitute for B-cells in promoting M-cell development (*Debard* et al., 1999; *Golovkina* et al., 1999). The role of lymphocytes in M-cell development is also supported by reports that co-culture of an intestinal epithelial cell-line (Caco-2) with Peyer's patch lymphocytes induces the epithelial cells to develop certain characteristics of M-cells (*Kernéis* et al., 1997). In this model B-lymphocyte cell lines, but not T-lymphocyte lines, were able to partially substitute for the Peyer's patch cells in inducing M-cell-like cells (*Kernéis* et al., 1997). Further analysis of knockout mice and *in vitro* models should permit characterisation of the factors stimulating M-cell development.

The finding that co-culture with lymphocytes converts Caco-2 cells into cells with characteristics of M-cells has been cited as evidence that differentiated enterocytes can also be transformed into M-cells. However, the fact that Caco-2 cells also retain crypt cell-like properties (e.g. the ability to divide) has led to others questioning whether similar transformations of enterocytes to M-cells occur *in vivo* (*Owen*, 1998b). Indeed, the bulk of evidence now indicates that the development of M-cells normally starts in the FAE-associated crypts. For example, lectin cytochemistry has shown that in mouse Peyer's patches and rabbit caecum, cells with characteristic lectin-binding signatures of M-cells are arranged in radial stripes within the FAE (*Clark* et al., 1993; *Gebert* and *Posselt*, 1997) and, in the mouse, emerge from morphologically specialised crypts (*Gebert* et al., 1999). These observations suggest that a proportion of the cells emanating from certain crypts is pre-programmed to develop into M-cells. Furthermore, a subpopulation of cells within FAE-associated crypts in rabbit caecum (*Gebert* and *Posselt*, 1997) and mouse Peyer's patch (Giannasca et al., 1994; Gebert et al., 1999) already displays lectin-binding patterns typical of mature M-cells. Despite these data we cannot exclude the possibility that, under appropriate conditions such as antigenic stimulation,

some enterocytes may retain an ability to differentiate into M-cells. For example, it has been reported that *Salmonella typhimurium* infection rapidly (12-24 h.) increases the population of alkaline phosphatase-negative cells throughout the FAE of mouse Peyer's patches, an observation consistent with new M-cells being formed from enterocytes (*Savidge* et al., 1991). Although this phenomenon has yet to be confirmed using positive cytochemical markers or functional definition of mouse M-cells, another recent report suggests that *Streptococcus pneumoniae* can induce rapid (within three hours) formation of M-cells in rabbit Peyer's patches (*Meynell* et al., 1999). In this study the presence of M-cells was inferred from measurement of increased microsphere binding and transcytosis following *Streptococcus* infection and tentatively confirmed by SEM (*Meynell* et al., 1999). *Borghesi* and co-authors (1999) subsequently confirmed by TEM analysis that newly formed M-cells appeared within one hour of *Streptococcus* infection. Interestingly, in this latter study newly formed M-cells were limited to the periphery of the dome suggesting the presence of an immature, uncommitted, epithelial cell population that retains the ability to switch to an M-cell phenotype. These observations on the development of M-cells have suggested the intriguing possibilities that M-cells may represent a highly regulated cell-population and that plasticity in the M-cell population might be exploited by pathogens (*Kernéis* and *Pringault*, 1999).

It is well established that cells with the morphological and functional characteristics of M-cells predominate in the periphery of rabbit and mouse FAE (e.g. *Savidge* et al., 1991; *Clark* et al., 1993; *Jepson* et al., 1993b,c; *Gebert* et al., 1996, 1999; *Gebert* and *Posselt*, 1997; *Borghesi* et al., 1999). The application of positive cytochemical markers has revealed that individual characteristics which are strongly expressed by peripheral M-cells disappear at different rates during maturation and migration to the dome apex (*Jepson* et al., 1993a; *Gebert* and *Posselt*, 1997), demonstrating that the M-cell phenotype is not static. It has also been suggested that the M-cell phenotype is transiently expressed and does not represent an end-point of epithelial cell differentiation. This latter view is supported by studies on mouse and chicken FAE which failed to detect apoptic cells displaying M-cell-specific cytochemical markers, apoptosis being restricted to the dome apex (*Debard* et al., 1999; *Takeuchi* et al., 1999; *Niedergang* and *Kraehenbuhl*, 2000). However, it should also be noted that other studies have not detected intermediate cell types close to the dome apex of rabbit Peyer's patch FAE: the presence of such cells would be expected if M-cells were to revert to enterocytes prior to their undergoing apoptosis and extrusion (*Borghesi* et al., 1999). Thus, significant controversies remain over the fate of M-cells, as is also the case with theories concerning the origin of M-cells and their relationship to FAE enterocytes. These issues have important implications for our understanding of the role of M-cells and the function and pathology of mucosa-associated lymphoid tissues.

INTERACTION OF MICRO-ORGANISMS WITH M-CELLS

As outlined previously, M-cells appear to be an important site of invasion by a diverse range of pathogenic bacteria, viruses and protozoa (*Gebert* et al., 1996; *Siebers* and *Finlay*, 1996; *Jepson* and *Clark*, 1998; *Sansonetti* and

Phalipon, 1999). Some pathogens, e.g. *Salmonella* and *Yersinia* (see below) selectively interact with M-cells while others, e.g. *Vibrio cholerae* exhibit tropism to the Peyer's patch FAE without clear preference for M-cells over enterocytes. In some species, including mice and rats, apathogenic segmented filamentous bacteria (SFBs) preferentially colonise Peyer's patch FAE, though without apparent preference for M-cells (*Klaasen* et al., 1992; *Jepson* et al., 1993d). In other species such as rabbit, the villi appear to be the preferred site of SFB attachment (*Heczko* et al., 2000a). Understanding the mechanisms by which micro-organisms selectively target and/or invade M-cells is a topic of considerable importance because it may ultimately permit the development of novel disease control strategies based on the prevention of initial pathogen invasion. In addition, it may prove possible to exploit microbial invasion pathways for mucosal drug and vaccine delivery.

Possible determinants of M-cell targeting and invasion

The study of cultured epithelial cells has provided a wealth of information on the adherence and invasion mechanisms employed by pathogenic bacteria (*Finlay* and *Cossart*, 1997; *Finlay* and *Falkow*, 1997). Such studies have revealed that bacteria employ diverse strategies to modulate host cell function involving, for example, intracellular signalling, cytoskeletal changes and cytokine release (*Finlay* and *Cossart*, 1997; *Finlay* and *Falkow*, 1997; *Wilson* et al., 1998). Some of these strategies are likely to be employed during the interaction of micro-organisms with M-cells, particularly since some of the events observed during bacterial/M-cell interactions appear to mimic the changes observed *in vitro*. However, it is also clear that additional mechanisms contribute to the interaction of micro-organisms with this specialised epithelial cell type. Some of these mechanisms may involve specific, microbial-mediated events whist others may be independent of active bacterial adhesive/invasive strategies and represent non-specific, M-cell-mediated transport. In recent years it has become increasingly clear that many pathogens, including *S. typhimurium* and *Yersinia* species possess multiple alternate mechanisms for M-cell invasion, a phenomenon which is perhaps unsurprising in view of the importance of the initial invasion step to the pathogenicity of these micro-organisms. Such a multiplicity of invasion mechanisms may permit the bacteria to invade the intestinal epithelial barrier under a wide variety of environmental and gut luminal conditions.

M-cells are able to transport inert particles (*Jepson* et al., 1993c,e, 1996; *Gebert* et al., 1996) and it has, therefore, been suggested that pathogens may similarly interact with M-cells via non-specific, passive mechanisms. Such mechanisms are likely to be influenced by the physical properties of the pathogen, as M-cell interactions with inert particles are influenced by the physicochemical properties of the particle preparations and also by species-related variations in M-cell surface properties (*Jepson* et al., 1993e, 1996). M-cell targeting by inert materials and bacteria may also be promoted by the reduced quantities of mucus overlying Peyer's patches and by the relatively poorly developed M-cell surface carbohydrate coat (glycocalyx) (*Gebert* et al., 1996; *Siebers* and *Finlay*, 1996; *Neutra* et al., 1999). Experiments with a range of cholera toxin B subunit conjugates have demonstrated that the glycocalyx acts as a size-selective barrier that limits access of particles, over a broad size range, to apical membrane glycolipids

of rabbit enterocytes. The reduced M-cell glycocalyx permits access of the smaller of these particles (sub-bacterial size) to the same membrane components (*Frey* et al., 1996). These data suggest that the M-cell glycocalyx, whilst thinner than that of enterocytes, may still limit the interaction between particles of bacterial size and the cell apical membranes. The precise effect of this phenomenon on bacterial interaction with cellular receptors is uncertain.

It is now clear that specific mechanisms also mediate M-cell targeting by micro-organisms, and recent studies have attempted to identify the bacterial and M-cell determinants responsible for these interactions. Several studies have examined whether bacterial proteins which mediate invasion of cultured cells also promote M-cell invasion. In some cases, these studies have demonstrated that modification of genes encoding *in vitro*-characterised invasion determinants significantly attenuates bacterial invasion via M-cells (*Jones* et al., 1994; *Sansonetti* et al., 1996; *Marra* and *Isberg*, 1997; *Penheiter* et al., 1997; *Clark* et al., 1998a,b; *Jepson* and *Clark*, 1998; *Sansonetti* and *Phalipon*, 1999). However, mutation of some bacterial genes has a less dramatic effect on M-cell invasion than that observed for cultured cells, indicating that multiple mechanisms contribute to M-cell entry. It is currently uncertain whether these include invasion mechanisms unique to the *in vivo* situation. The bacterial proteins which mediate M-cell targeting have now been identified for some bacterial species, although the identity of the corresponding M-cell surface receptors remains less certain. It has been hypothesised that components of the M-cell glycocalyx mediate M-cell targeting by micro-organisms, and that the site- and species-related variations in this glycocalyx may contribute to tissue tropism exhibited by pathogens (*Clark* et al., 1994b; *Giannasca* et al., 1994; *Jepson* and *Clark*, 1998; *Neutra* et al., 1999). This hypothesis awaits rigorous investigation. Interestingly, recent studies have investigated whether inert materials (e.g. vaccine preparations) might be targeted to M-cells using ligands that selectively bind to M-cell-specific surface carbohydrates (*Clark* et al., 2000). This approach has proved successful for targeting inert particles and/or macromolecules for uptake by M-cells in mouse Peyer's patches (*Giannasca* et al., 1994; *Clark* et al., 1995; *Chen* et al., 1996; *Foster* et al., 1998) and hamster nasal lymphoid tissue (*Giannasca* et al., 1997).

Salmonella

The interaction of *Salmonella typhimurium* with intestinal M-cells has received considerable attention in recent years, due in part to the potential importance of this bacterium as a live oral vaccine delivery vehicle. Studies utilising a mouse gut loop model have demonstrated that *S. typhimurium* selectively targets to and invades mouse M-cells (*Clark* et al., 1994a; *Jones* et al., 1994). Invasion of murine M-cells is accompanied by M-cell destruction and sloughing of the FAE (*Jones* et al., 1994; *Pascopella* et al., 1995; *Clark* et al., 1998b; *Jensen* et al., 1998; *Jepson* and *Clark*, 1998). *S. typhimurium* infection of calf ileal loops similarly results in rapid invasion and destruction of M-cells and is accompanied by widespread FAE damage (*Frost* et al., 1997). The epithelial damage induced by *Salmonella* is likely to have serious consequences in naturally infected animals as it will result in unrestricted bacterial invasion and may explain the frequent occurrence of intestinal ulcerations and perforation in typhoid patients. In addition, it is essential that *Salmonella*-based oral vaccines are designed to minimise epithelial damage.

It has been demonstrated in the mouse gut loop model that the extent of *Salmonella* invasion and associated epithelial damage is dependent on the composition of the inoculum. Bacteria suspended in Luria-Bertani (LB) broth initiated extensive FAE damage, whereas damage was negligible when bacteria were inoculated in phosphate-buffered saline (*Clark* et al., 1998b). These findings suggest that local environmental factors within the gut lumen may influence the course of *Salmonella* infections. The regulation of *Salmonella* invasion and FAE destruction is not well understood, although one study has indicated that FAE destruction, but not M-cell invasion, is promoted by the regulator SlyA (*Daniels* et al., 1996). It has also been reported that mutants lacking DNA adenine methylase fail to evoke significant M-cell cytotoxicity - a property which might contribute to their enhanced ability to promote immune responses (*Garcia-del Portillo* et al., 1999; *Heithoff* et al., 1999).

The mechanisms responsible for M-cell invasion by *Salmonella* await precise identification, although a number of factors that may contribute to this process have been identified. For example, the invasion machinery encoded by *Salmonella* pathogenicity island 1 (SPI1) which is essential for the efficient invasion of cultured cell lines is also one of the factors that contributes to M-cell invasion by *S. typhimurium* (*Jones* et al., 1994; *Penheiter* et al., 1997; *Clark* et al., 1998b). When inoculated in LB, SPI1-deficient *S. typhimurium* mutants exhibit reduced levels of M-cell invasion compared with wild-type bacteria (*Clark* et al., 1998b). However, these mutants still invade M-cells in significant numbers (*Clark* et al., 1996, 1998b), and M-cell invasion is accompanied by membrane remodelling (ruffling) similar to that induced by wild-type *S. typhimurium* both *in vitro* and *in vivo*. Consistent with these observations, SPI1 mutants readily kill mice following oral infection although they exhibit 10-100-fold higher oral LD_{50} values than wild-type organisms (*Penheiter* et al., 1997). Interestingly, SPI1 mutants consistently fail to induce M-cell damage or FAE destruction (*Jones* et al., 1994, *Penheiter* et al., 1997; *Clark* et al., 1998b). At present it is uncertain whether this phenomenon is solely a consequence of insufficient bacterial invasion or whether SPI1-encoded proteins also have a direct role in the destructive process.

It has been suggested that M-cell targeting by *S. typhimurium* is mediated by a specific adhesin, namely the long polar fimbria (LPF) encoded by the *lpf* operon (*Bäumler* et al., 1996). This proposal was initially based on the observation that *lpfC* mutants exhibit reduced colonisation of Peyer's patches and moderately increased oral LD_{50} values compared with wild-type bacteria. Further evidence that LPF may target bacteria to M-cells came from the finding that incorporation of the *lpf* operon into non-piliated *Escherichia coli* enhanced their uptake into Peyer's patches (*Bäumler* et al., 1996) and from observations on the phase variation of the *lpf* operon which demonstrated that greater numbers of *S. typhimurium* recovered from Peyer's patches were of the phase-on phenotype (*Norris* et al., 1998). It is clear, however, that LPF is not the sole adhesin involved in *Salmonella* infection, as strains carrying mutations in *lpfC* or both *lpfC* and *invA* (a critical component of SPI1) still kill mice, albeit with increased oral LD_{50} values (*Bäumler* et al., 1996, 1997). Consistent with this observation, our own preliminary studies have demonstrated that *S. typhimurium* strains carrying mutations in *lpfC* or both *lpfC* and *invA* still enter murine M-cells (*Jepson* and *Clark*, 1998), and recent studies have also

demonstrated that mutation of *lpfC* has no measurable effect on the mucosal immune response to *S. typhimurium* following oral inoculation (*Vazquez-Torres* et al., 1999). Furthermore, the distribution of the *lpf* operon is not closely correlated with the ability of Salmonella strains to invade M-cells in murine gut loops, because it is absent from *Salmonella typhi*, which invades murine M-cells in this model, and is present in *Salmonella gallinarium*, which does not (*Pascopella* et al., 1995; *Bäumler* et al., 1997). Multiple fimbrial operons contribute to *Salmonella* virulence (*van der Velden* et al., 1998), and it is now clear that neither the *lpf* operon nor SPI1 are the sole determinants of intestinal invasion by *S. typhimurium*. It is perhaps not surprising that there appears to be a degree of redundancy in the mechanisms employed by *Salmonella* to enter M-cells and to breach the intestinal epithelial barrier since this is arguably the most critical event contributing to *Salmonella* virulence.

It must be noted that M-cells constitute a minute proportion of the intestinal epithelia (estimated at around 0.01%). The intestinal surface area available for bacterial translocation will, however, exhibit a localised increase if, as in the case of *Salmonella* infection, M-cell invasion is accompanied by FAE destruction. In addition, extra-intestinal dissemination of enteropathogens such as *Salmonella* is also likely to involve translocation at other non-FAE sites. Indeed, the first reports of *Salmonella* invasion of gut epithelia described entry into guinea pig enterocytes (*Takeuchi*, 1967) and subsequent reports in species other than mice have consistently drawn attention to enterocyte invasion (*Wallis* et al., 1986; *Frost* et al., 1997; *Bolton* et al., 1999a,b). Recently it has been proposed that an M-cell-independent route of extra-intestinal dissemination of *Salmonella* and possibly other enteropathogens is mediated by CD18-expressing phagocytes. It has been suggested that these phagocytes may either engulf luminal bacteria at the apical epithelial surfaces or engulf bacteria which have gained access to the lamina propria via paracellular pathways (*Vazquez-Torres* et al., 1999; *Vazquez-Torres* and *Fang*, 2000). The proposed role of this CD18-mediated route was based on the observation that, after oral administration, dissemination to spleen and liver by an attenuated *S. typhimurium* mutant (lacking *aroA*, *lpfC*, *invA*) was reduced in CD18 knockout mice compared to congenic controls. In addition, following inoculation of ligated ileal loops, invasion deficient (lacking *aroA*, *invA*) bacteria expressing green fluorescent protein (GFP) were observed within CD18-expressing phagocytes located in the villous lamina propria (*Vazquez-Torres* et al., 1999). It was further proposed that the ability of SPI1 deficient mutants to elicit a serum IgG (but not a mucosal IgA) response might be a consequence of CD18-mediated bacterial dissemination in the absence of the M-cell mediated bacterial uptake required for a mucosal response (*Vazquez-Torres* et al., 1999). The implication that M-cells play no part in this CD18-mediated route was based on the assumption that *lpfC/invA* mutants cannot enter M-cells and seems somewhat premature since the possible existence of such a route was not investigated. Indeed, our preliminary data have demonstrated that *lpfC/invA* mutant *S. typhimurium* can enter murine M-cells following infection of gut loops (*Jepson* and *Clark*, 1998), and it therefore remains possible that M-cells contribute to the extra-intestinal dissemination reported by *Vazquez-Torres* and co-authors (1999).

Yersinia

Early studies suggested that M-cells are, at least during the early stages of infection, a major site of invasion by the enteropathogenic *Yersinia* species (*Hanski* et al., 1989; *Fujimura* et al., 1992). Detailed microscopic studies have now demonstrated that murine M-cells are preferentially invaded by *Yersinia pseudotuberculosis* (Figure 1; *Clark* et al., 1998a) and *Yersinia enterocolitica* (*Autenrieth* and *Firsching*, 1996), and that infection with the latter may result in severe damage to the Peyer's patches (*Autenrieth* and *Firsching*, 1996). The mechanisms responsible for M-cell targeting by *Y. pseudotuberculosis* have been partially defined. As with the infection of cultured cell lines (*Isberg* and *Leong*, 1990), interaction of *Y. pseudotuberculosis* with intestinal M-cells is primarily mediated by the bacterial protein invasin, which promotes both M-cell adhesion and invasion (*Marra* and *Isberg*, 1997; *Clark* et al., 1998a). In contrast to enterocytes, murine Peyer's patch M-cells express β_1 integrins on their apical surfaces (*Clark* et al., 1998a) and, since multiple β_1 integrins act as receptors for invasin-mediated interactions with cultured cell lines (*Isberg* and *Leong*, 1990), it seems likely that M-cell surface β_1 integrins similarly act as receptors for *Yersinia in vivo*. This hypothesis awaits rigorous investigation. In support of a more widespread role for β_1 integrins in M-cell targeting by *Yersinia*, it was recently reported that expression of these receptors on the apical surface of a human epithelial cell line (Caco-2) is increased by co-culture with lymphocytes (*Schulte* et al., 2000). Furthermore, *Y. enterocolitica* were shown to utilise β_1 integrin receptors in this *in vitro* model of M-cell infection (*Schulte* et al., 2000). Together these findings suggest that M-cell surface integrins may act as receptors for the *in vivo* invasion of *Yersinia* or other pathogens in a variety of host species and mucosal sites in addition to mouse Peyer's patches. This hypothesis currently awaits thorough investigation.

It is clear that the enteropathogenic *Yersinia* species also possess invasin-independent routes of intestinal invasion. This is supported by the observations that invasin deficient mutants of *Y. pseudotuberculosis* retain the capacity to adhere to and invade M-cells, albeit at dramatically reduced levels (*Clark* et al., 1998a), and to colonise the Peyer's patches (*Marra* and *Isberg*, 1997) and by results obtained using the Caco-2 cell/lymphocyte co-culture model of *Yersinia* infection (*Schulte* et al., 2000). Gut loop infection with mutants deficient in two other *in vitro* identified adhesins, namely YadA and the pH 6 antigen, failed to detect a direct role for either protein in M-cell invasion by *Y. pseudotuberculosis* (*Marra* and *Isberg*, 1997). It thus appears that *Yersinia* invasion *in vivo* can proceed via additional mechanisms to those previously identified in conventional cultured cell lines. The mechanisms and significance of these alternative mechanisms of *Yersinia* invasion remain unclear, as does the possible contribution made by enterocytes to intestinal invasion by *Yersinia* species.

Enteropathogenic and enterohaemorrhagic Escherichia coli

Intestinal infection by enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) strains involve the formation of attaching and effacing (A/E) lesions, with EPEC adhering intimately to membrane pedestals under which cytoskeletal proteins accumulate (*Celli* et al., 2000). The rabbit EPEC (REPEC) strain RDEC-1, adheres selectively to rabbit Peyer's patch M-cells (*Cantey* and *Inman*, 1981; *Inman* and *Cantey*, 1984) and, in striking contrast

to other pathogens and inert particles which are readily taken up by M-cells following initial adherence, RDEC-1 adherence to M-cells does not result in their internalisation (*Inman* and *Cantey*, 1984). At present, it is unclear how RDEC-1 resists M-cell phagocytosis, although this is probably related to the ability of EPEC strains to hijack host signalling pathways and induce cytoskeletal reorganisation (*Celli* et al., 2000).

RDEC-1 is also unusual among M-cell-selective pathogens in that the primary adhesin mediating M-cell targeting, the AF/R1 pilus, has been recognised for some time (*Inman* and *Cantey*, 1984). While the distribution of REPEC binding between FAE and villous epithelium varies between strains, with FAE binding being favoured by all AF/R1 pilus-expressing strains examined in detail, REPEC strains lacking AF/R1 pili do adhere to Peyer's patch FAE, indicating that this is not the only adhesin that can mediate FAE binding (*von Moll* and *Cantey*, 1997). Indeed, Heczko and co-authors (2000b) recently reported that REPEC O103 strain 85/150, which expresses different (AF/R2) pili, also preferentially colonise rabbit Peyer's patch FAE in the early stages of infection although it is unclear whether this phenomenon involves M-cell tropism. Interestingly, Heczko and co-authors have also reported that commensal SFBs, which had previously not been described in rabbits, prevented ileal colonisation and the induction of clinical disease by REPEC O103 (*Heczko* et al., 2000a). Similarly, circumstantial evidence suggests that SFBs may also exert a negative effect on ileal colonisation by *Salmonella* in rats (*Garland* et al., 1982). The mechanisms by which SFBs may protect against enteropathogens remain unclear (*Klaasen* et al., 1992), although it has been suggested that they may exert their influence by mechanical exclusion since massive numbers of SFBs are frequently located in the distal ileum, a site favoured by many enteropathogens including *Salmonella* (*Garland* et al., 1982). SFBs must also exert their influence by additional, as yet undefined mechanisms since REPEC O103 colonises the FAE of the rabbit distal ileum, whereas colonisation by rabbit SFBs appears to be restricted to the villi (*Heczko* et al., 2000a). It is possible that SFBs may induce colonisation resistance by stimulating the mucosal immune system (*Klaasen* et al., 1993) or by modifying local environmental conditions within the gut lumen.

The study of rabbit EPEC strains has suggested that human EPEC and/or EHEC might also target Peyer's patch M-cells or FAE but until recently this possibility remained untested. Elegant studies employing *in vitro* organ culture (IVOC) of human intestine have now examined initial interactions of EPEC and EHEC with various regions of paediatric intestine (*Phillips* et al., 2000). These studies revealed that the EPEC strain E2348/69 adhered to both proximal and distal small intestine and to Peyer's patch FAE while, in marked contrast, the EHEC O157:H7 strain 85-170 adhered only to Peyer's patch FAE during the time-course of these experiments. The extent of adherence of EHEC suggested that this involves enterocytes, and the possible involvement of M-cells could not be inferred from these studies. *Phillips* and co-authors (2000) hypothesised that EHEC intimin might be responsible for the preferential binding to FAE since EHEC express a different form of intimin (γ) to that expressed by EPEC E2348/69 (intimin-α). Confirmation of the role of intimin in EPEC/EHEC tissue-specificity has subsequently been provided by infection of human IVOC with EPEC E2348/69 after replacement of the eaeα gene

(encoding intimin-α) with the EHEC eaeγ gene (intimin-γ) and by a natural intimin-γ-expressing EPEC O55:H7 strain; both of which exhibited highly selective binding to Peyer's patch FAE (Phillips and Frankel, 2000). While these data indicate that intimin does mediate FAE binding the cellular receptor remains unidentified, though since intimin is known to bind β1 integrins it is tempting to speculate that these molecules might play a role in EHEC/EPEC tissue tropism.

Shigella flexneri

Shigellosis is characterised by acute recto-colitis, and is caused by members of the genus *Shigella* invading the intestinal epithelium. *In vitro* studies have demonstrated that *Shigella* invasion of intestinal epithelial cell lines is restricted to their basolateral surfaces, and it now appears that *Shigella* invasion *in vivo* initially occurs via M-cells and at sites where epithelial integrity is compromised (*Sansonetti* and *Phalipon*, 1999). The putative role of M-cells in initial *Shigella* invasion is supported by the observations that early lesions in patients and Macaque monkeys infected with *Shigella* are located over the lymphoid follicles and from experimental studies using the rabbit model of infection (*Sansonetti* and *Phalipon*, 1999). The latter studies have demonstrated that *Shigella flexneri* selectively invades M-cells in rabbit gut loops (*Wassef* et al., 1989; *Perdomo* et al., 1994). As with *S. typhimurium*, M-cell infection by *S. flexneri* is associated with membrane remodelling similar to that seen following invasion of cultured cells (*Sansonetti* et al., 1996). In contrast to *S. typhimurium* infection, *S. flexneri* does not appear to exert a direct cytotoxic effect on M-cells. However, by 8 h. post infection, *S. flexneri* induces a massive inflammatory response which results in extensive FAE destruction (*Perdomo* et al., 1994; *Sansonetti* et al., 1996). Analysis of mutant strains revealed that both an adhesive and an invasive phenotype were required for efficient FAE colonisation (*Sansonetti* et al., 1996). Mutant strains deficient for these phenotypes induced only a limited inflammatory response, and this permitted the cellular changes associated with *Shigella* infection to be analysed in more detail (*Sansonetti* et al., 1996). An invasion deficient mutant, cured of a virulence plasmid essential for invasion of cultured cells, was rendered M-cell adherent by expression of the RDEC-1 M-cell adhesin (AF/R1 pili) (*Inman* and *Cantey*, 1984; *Sansonetti* et al., 1996). Infection with this mutant induced migration of mononuclear cells into the M-cell pockets, which was accompanied by a marked increase in the proportion of the FAE occupied by M-cells (*Sansonetti* et al., 1996). This increase in M-cell surface area resulted from an enlargement of individual cells rather than from an increase in their number as has been reported after *Salmonella* infection of mice (*Savidge* et al., 1991) and *Streptococcus* infection of rabbit Peyer's patches (*Borghesi* et al., 1999; *Meynell* et al., 1999).

The interaction of *S. flexneri* in mouse gut loops has now also been described (*Jensen* et al., 1998). During early infection bacteria are associated with the surface of mouse Peyer's patch M-cells, with some M-cells exhibiting surface protrusions. Extensive FAE destruction is apparent 90 min post infection, although it is unclear whether this is a direct consequence of bacterial invasion or, as in the rabbit, a result of bacterial-induced inflammatory responses. Relatively large numbers ($>10^9$ CFU/ml) of *S. flexneri* are required to induce FAE destruction compared to those required for *S. typhimurium*-induced cytotoxicity, confirming that this *Salmonella*-induced cytotoxic response

is distinct from responses induced by other pathogens.

Listeria monocytogenes

Some studies have suggested that, following oral infection of mice with *Listeria monocytogenes*, Peyer's patches constitute the primary site of bacterial infection (*Marco* et al., 1992). These observations suggest that *Listeria* might be added to the list of pathogens that utilise M-cells as a gateway into the host (*Gebert* et al., 1996). However, some studies were unable to demonstrate involvement of M-cells in *L. monocytogenes* infection following oral infection of mice (*Marco* et al., 1997) or inoculation of rat gut loops (*Pron* et al., 1998), and it has been suggested that Peyer's patches may instead serve as a preferential site of *Listeria* replication rather than invasion (*Pron* et al., 1998). In contrast to these findings, interaction between *L. monocytogenes* and mouse M-cells has been observed in gut loops infected with large numbers (>10^9 CFU/ml) of bacteria (*Jensen* et al., 1998). In this latter study, *L. monocytogenes* was also reported to induce extensive loss of FAE-cells although much larger inoculae of this bacterium were required to induce FAE damage than that required for *S. typhimurium* (*Jensen* et al., 1998). The inability of some studies to demonstrate a clear role for M-cells in *Listeria* infection suggest the existence of M-cell-independent route(s) of invasion. Recent studies have examined this possibility and demonstrated extra-intestinal dissemination of *L. monocytogenes* after intragastric inoculation of germfree severe-combined immunodeficient (SCID) mice and after rectal inoculation of normal mice (*Havell* et al., 1999). These observations clearly demonstrate that *L. monocytogenes* can translocate across the intestine where fully formed Peyer's patches are absent. However, this approach does not exclude the possible involvement of small numbers of M-cells in the observed translocation since these cells are present in the colon of conventional mice (*Giannasca* et al., 1994; *Gebert* et al., 1996) and in rudimentary GALT of SCID mice (*Savidge*, 1996). Further research is thus required to fully elucidate the role of M-cells, if any, in natural *Listeria* infections.

DISCUSSION

Significant progress has been made in recent years in our understanding of M-cell biology and function. However, despite these advances, many questions remain unresolved and it is hoped that the continuing refinement of *in vitro* and *in vivo* experimental tools will facilitate further characterisation of these fascinating cells. The bulk of experimental evidence now indicates that M-cells primarily originate from undifferentiated crypt cells under the influence of local lymphoid factors. However, the possibility that M-cells may represent a dynamic cell population which can rapidly respond to changes in the local levels of antigenic stimulation is fascinating and merits further investigation. Future analyses should investigate more closely the possibility that enterocytes may differentiate into M-cells, identify the range of micro-organisms which might induce such an event and determine the functional status of these newly formed M-cells. Further studies are also required to determine the fate of M-cells.

Accumulating evidence from a range of animal species now indicates that many pathogens exploit M-cells as a

route of host invasion. In recent years significant progress has been made towards characterising the mechanisms by which these pathogens interact with M-cells and in defining the consequences of these interactions. Much, however, remains to be learnt. It is already clear that bacteria employ a range of strategies to subvert M-cell function and to exploit these cells as a gateway into the host or, in the case of EPEC, to resist internalisation. In some cases, these strategies mimic those previously identified *in vitro* (*Marra* and *Isberg*, 1997; *Penheiter* et al., 1997; *Clark* et al., 1998a,b) but it is also clear that additional, previously unidentified, mechanisms contribute to M-cell invasion. These studies highlight the fact that there is some degree of redundancy in the mechanisms employed by many pathogens to breach the intestinal epithelial barrier and that individual pathogens may encode a variety of alternate mechanisms to exploit M-cell function. Similarly, there appears to be redundancy in the bacterial ligands and cellular receptors that mediate adherence to M-cells. In many cases the precise nature of these bacterial adhesins, cellular receptors and invasion mechanisms await further investigation, as does the relative contribution of these mechanisms to bacterial infectivity.

It is now recognised that many of the pathogens which invade M-cells also induce significant changes in Peyer's patch architecture including alterations in M-cell morphology or number, cellular infiltration and epithelial destruction ranging from localised M-cell disruption to complete loss of the FAE. It is not yet clear what factors regulate these responses and whether they are analogous to changes observed upon infection of cultured cells. However, the observed changes suggest that bacterial interaction with M-cells may also play a key role in the pathogenesis of important human diseases, including typhoid fever, shigellosis and Crohn's disease (*Siebers* and *Finlay*, 1996; *Sansonetti* et al., 1996), although direct evidence of the significance of M-cell infection in human disease is currently lacking. It is clear, however, that M-cells play a key role in both infection and immunity in animal infection models. It is less clear what factors determine whether microbial internalisation by M-cells results in the induction of a protective immune response or the generation of local or systemic disease. It should, however, be remembered that, while M-cells appear to represent a major route of pathogen invasion, they constitute only a minute proportion of the mucosal surface area and thus bacterial translocation may also be significant through other less efficient routes. Similarly, while M-cells are the specialised antigen-sampling cells of the mucosal immune system, other cell types also appear to contribute to this function. Much further research is still required to complete our understanding of M-cell/micro-organism interactions. Such understanding is of considerable importance, since further definition of the nature of these interactions is likely to prove invaluable in the design of mucosal vaccines and the development of effective disease control strategies against pathogens that colonise or invade mucosal surfaces.

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LITERATURE

Autenrieth, I.B., and Firsching, R.: Penetration of M cells and destruction of Peyer's patches by *Yersinia enterocolitica* : an ultrastructural and histological study. J. Med. Microbiol. 44, 285-294 (1996).

Bäumler, A.J., Tsolis, R.M., and Heffron, F.: The lpf fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. Proc. Natl. Acad. Sci. (USA) 93, 279-283 (1996).

Bäumler, A.J., Tsolis, R.M., Valentine, P.J., Ficht, T.A., and Heffron, F.: Synergistic effect of mutations in *invA* and *lpfC* on the ability of *Salmonella typhimurium* to cause murine typhoid. Infect. Immun. 65, 2254-2259 (1997).

Bolton, A.J., Martin, G.D., Osborne, M.P., Wallis, T.S., and Stephen, J.: Invasiveness of *Salmonella* serotypes Typhimurium, Choleraesuis and Dublin for rabbit terminal ileum *in vitro*. J. Med. Microbiol. 48, 1-10 (1999a).

Bolton, A.J., Osborne, M.P., Wallis, T.S., and Stephen, J.: Interaction of *Salmonella choleraesuis, Salmonella dublin* and *Salmonella typhimurium* with porcine and bovine terminal ileum *in vivo*. Microbiol. 145, 2431-2441 (1999b).

Borghesi, C., Taussig, M.J., and Nicoletti, C.: Rapid appearance of M cells after microbial challenge is restricted at the periphery of the follicle-associated epithelium of Peyer's patch. Lab. Invest. 79, 1393-1401 (1999).

Cantey, J.R., and Inman, L.R.: Diarrhea due to *Escherichia coli* strain RDEC-1 in the rabbit: The Peyer's patch as the initial site of attachment and colonization. J. Infect. Dis. 143, 440-446 (1981).

Celli, J., Deng, W., and Finlay, B.B.: Enteropathogenic *Escherichia coli* (EPEC) attachment to epithelial cells: exploiting the host cell cytoskeleton from the outside. Cellular Microbiol. 2, 1-9 (2000).

Chen, H., Torchilin, V., and Langer, R.: Lectin-bearing polymerized liposomes as potential oral vaccine carriers. Pharm. Res. 13, 1378-1383 (1996).

Clark, M.A., Jepson, M.A., Simmons, N.L., Booth, T.A., and Hirst, B.H.: Differential expression of lectin-binding sites defines mouse intestinal M-cells. J. Histochem. Cytochem. 41, 1679-1687 (1993).

Clark, M.A., Jepson, M.A., Simmons, N.L., and Hirst, B.H.: Preferential interaction of *Salmonella typhimurium* with mouse Peyer's patch M cells. Res. Microbiol. 145, 543-552 (1994a).

Clark, M.A., Jepson, M.A., Simmons, N.L., and Hirst, B.H.: Differential surface characteristics of M cells from mouse intestinal Peyer's and caecal patches. Histochem. J. 26, 271-280 (1994b).

Clark, M.A., Jepson, M.A., Simmons, N.L., and Hirst, B.H.: Selective binding and transcytosis of *Ulex europaeus* 1 lectin by mouse Peyer's patch M-cells *in vivo*. Cell Tissue Res. 282, 455 - 461 (1995).

Clark, M.A., Reed, K.A., Lodge, J., Stephen, J., Hirst, B.H., and Jepson, M.A.: Invasion of murine intestinal M cells by *Salmonella typhimurium inv* mutants severely deficient for invasion of cultured cells. Infect. Immun. 64, 4363-4368 (1996).

Clark, M.A., Hirst, B.H., and Jepson, M.A.: M-cell surface β1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. Infect. Immun. 66, 1237-1243 (1998a).

Clark, M.A., Hirst, B.H., and Jepson, M.A.: Inoculum composition and *Salmonella* pathogenicity island 1 regulate M-cell inva-

sion and epithelial destruction by *Salmonella typhimurium.* Infect. Immun. 66, 724-731 (1998b).

Clark, M.A., Hirst, B.H., and Jepson, M.A.: Lectin-mediated mucosal delivery of drugs and microparticles. Adv. Drug Deliv. Rev. 43, 207-223 (2000).

Daniels, J.J.D., Autenrieth, I.B., Ludwig, A., and Goebel, W. The gene *slyA* of *Salmonella typhimurium* is required for destruction of M cells and intracellular survival but not for invasion or colonization of the murine small intestine. Infect. Immun. 64, 5075-5084 (1996).

Debard, N., Sierro, F., and Kraehenbuhl, J.P.: Development of Peyer's patches, follicle-associated epithelium and M cell: lessons from immunodeficient and knockout mice. Semin. Immunol. 11, 183-191 (1999).

Finlay, B.B., and Cossart, P.: Exploitation of mammalian host cell functions by bacterial pathogens. Science 276, 718-725 (1997).

Finlay, B.B., and Falkow, S.: Common themes in microbial pathogenicity revisited. Microbiol. Mol. Biol. Rev. 61, 136-169 (1997).

Foster, N., Clark, M.A., Jepson, M.A., and Hirst, B.H.: *Ulex europaeus* 1 lectin targets microspheres to mouse Peyer’s patch M-cells *in vivo*. Vaccine 16, 536 - 541 (1998).

Frey, A., Giannasca K.T., Weltzin R., Giannasca, P.J., Reggio, H., Lencer W.I., and Neutra, M.R.: Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: Implications for microbial attachment and oral vaccine targeting. J. Exp. Med. 184, 1045-1059 (1996).

Frost, A.J., Bland, A.P., and Wallis, T.S.: The early dynamic response of the calf ileal epithelium to *Salmonella typhimurium.* Vet. Pathol. 34, 369-386 (1997).

Fujimura, Y., Kihara, T., and Mine, H.: Membranous cells as a portal of *Yersinia pseudotuberculosis* entry into rabbit ileum. J. Clin. Electron Microscopy 25, 35-45 (1992).

García-del Portillo, F., Pucciarelli, M.G., and Casadesús, J.: DNA adenine methylase mutants of *Salmonella typhimurium* show defects in protein secretion, cell invasion, and M cell cytotoxicity. Proc. Natl. Acad. Sci. USA. 96, 11578-11583 (1999).

Garland, C.D., Lee, A., and Dickson, M.R.: Segmented filamentous bacteria in the rodent small intestine: their colonization of growing animals and possible role in host resistance to *Salmonella*. Microb. Ecol. 8, 181-190 (1982).

Gebert, A.: The role of M cells in the protection of mucosal membranes. Histochem. Cell Biol. 108, 455-470 (1997).

Gebert, A., and Hach, G.: Differential binding of lectins to M cells and enterocytes in the rabbit cecum. Gastroenterology 105, 1350-1361 (1993).

Gebert, A., and Pabst, R.: M cells at locations outside the gut. Semin. Immunol. 11, 165-170 (1999).

Gebert, A., and Posselt, W.: Glycoconjugate expression defines the origin and differentiation pathway of intestinal M-cells. J. Histochem. Cytochem. 45, 1341-1350 (1997).

Gebert, A., Hach, G., and Bartels, H.: Co-localization of vimentin and cytokeratins in M cells of rabbit gut-associated lymphoid tissue (GALT). Cell Tissue Res. 269, 331-340 (1992).

Gebert, A., Rothkötter, H-J., and Pabst, R.: Cytokeratin 18 is an M cell-marker in porcine Peyer’s patches. Cell Tissue Res. 276, 213-221 (1994).

Gebert, A., Rothkötter, H-J., and Pabst, R.: M cells in Peyer’s patches of the intestine. Int. Rev. Cytol. 167, 91-159 (1996).

Gebert, A., Fassbender, S., Werner, K., and Weissferdt, A.: The development of M cells in Peyer’s patches is restricted to specialized dome-associated crypts. Am. J. Pathol. 154, 1573-1582 (1999).

Giannasca, P.J., Giannasca, K.T., Falk, P., Gordon, J.I., and Neutra, M.R.: Regional differences in glycoconjugates of intestinal M cells in mice: potential targets for mucosal vaccines. Am. J. Physiol. 267, G1108-G1121 (1994).

Giannasca P.J., Boden, J.A., and Monath, T.P.: Targeted delivery of antigen to hamster nasal lymphoid tissue with M-cell-directed lectins. Infect. Immun. 65, 4288 – 4298 (1997).

Giannasca, P.J., Giannasca, K.T., Leichtner, A.M., and Neutra, M.R.: Human intestinal M cells display the sialyl Lewis A antigen. Infect. Immun. 67, 946-953 (1999).

Golovkina, T.V., Shlomchik, M., Hannum, L., and Chervonsky, A.: Organogenic role

of B lymphocytes in mucosal immunity. Science. 286, 1965-1968 (1999).

Hanski, C., Kutschka, U., Schmoranzer, H.P., Naumann, M., Stallmach, A., Hahn, H., Menge, H., and Riecken, E.O.: Immunohistochemical and electron microscopic study of interaction of *Yersinia enterocolitica* serotype O8 with intestinal mucosa during experimental enteritis. Infect. Immun. 57, 673-678 (1989).

Havell, E.A., Beretich, G.R., and Carter, P.B.: The mucosal phase of *Listeria* infection. Immunobiol. 201, 164-177 (1999).

Heczko, U., Abe, A., and Finlay, B.B.: Segmented filamentous bacteria prevent colonization of enteropathogenic Escherichia coli O103 in rabbits. J. Infect. Dis. 181, 1027-1033 (2000a).

Heczko, U., Abe, A., and Finlay, B.B.: *In vivo* interactions of rabbit enteropathogenic *Escherichia coli* O103 with its host: an electron microscopic and histopathologic study. Microbes Infect. 2, 5-16 (2000b).

Heithoff, D.M., Sinsheimer, R.L., Low, D.A., and Mahan, M.J.: An essential role for DNA adenine methylation in bacterial virulence. Science 284, 967-970 (1999).

Inman, L.R., and Cantey, J.R.: Peyer's patch lymphoid follicle epithelial adherence of a rabbit enteropathogenic *Escherichia coli* (Strain RDEC-1). J. Clin. Invest. 74, 90-95 (1984).

Isberg, R.R., and Leong, J.M.: Multiple β_1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. Cell 60, 861-871 (1990).

Jensen, V.B., Harty, J.T., and Jones, B.D.: Interactions of the invasive pathogens *Salmonella typhimurium*, *Listeria monocytogenes*, and *Shigella flexneri* with M cells and murine Peyer's patches. Infect. Immun. 66, 3758-3766 (1998).

Jepson, M.A., and Clark, M.A.: Studying M cells and their role in infection. Trends Microbiol. 6, 359-365 (1998).

Jepson, M.A., Mason, C.M., Bennett, M.K., Simmons, N.L., and Hirst, B.H.: Co-expression of vimentin and cytokeratins in M cells of rabbit intestinal lymphoid follicle-associated epithelium. Histochem. J. 24, 33-39 (1992).

Jepson, M.A., Clark, M.A., Simmons, N.L., and Hirst, B.H.: Epithelial M cells in the rabbit caecal lymphoid patch display distinctive surface characteristics. Histochemistry 100, 441-447 (1993a).

Jepson, M.A., Simmons, N.L., Hirst, G.L., and Hirst, B.H.: Identification of M cells and their distribution in rabbit intestinal Peyer's patches and appendix. Cell Tissue Res. 273, 127-136 (1993b).

Jepson, M.A., Simmons, N.L., Savidge, T.C., James, P.S., and Hirst, B.H.: Selective binding and transcytosis of latex microspheres by rabbit intestinal M cells. Cell Tissue Res. 271, 399-405 (1993c).

Jepson, M.A., Clark, M.A., Simmons, N.L., and Hirst, B.H.: Actin accumulation at sites of attachment of indigenous apathogenic segmented filamentous bacteria to mouse ileal epithelial cells. Infect. Immun. 61, 4001-4004 (1993d).

Jepson, M.A., Simmons, N.L., O'Hagan, D.T., and Hirst, B.H.: Comparison of poly(DL-lactide-co-glycolide) and polystyrene microsphere targeting to intestinal M cells. J. Drug Targeting 1, 245 - 249 (1993e).

Jepson, M.A., Mason, C.M., Clark, M.A., Simmons, N.L., and Hirst, B.H.: Variations in lectin binding properties of intestinal M cells. J. Drug Targeting 3, 75-77 (1995a).

Jepson, M.A., Mason, C.M., Simmons, N.L., and Hirst, B.H.: Enterocytes in the follicle-associated epithelia of rabbit small intestine display distinctive lectin-binding properties. Histochemistry 103, 131 - 134 (1995b).

Jepson, M.A., Clark, M.A., Foster, N., Mason, C.M., Bennett, M.K., Simmons, N.L., and Hirst, B.H.: Targeting to intestinal M cells. J. Anat. 189, 507-516 (1996).

Jones, B.D., Ghori, N., and Falkow, S.: *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. J. Exp. Med. 180, 15-23 (1994).

Kernéis, S., and Pringault, E.: Plasticity of the gastrointestinal epithelium: The M cell paradigm and opportunism of pathogenic microorganisms. Semin. Immunol. 11, 205-215 (1999).

Kernéis, S., Bogdanova, A., Kraehenbuhl, J.P., and Pringault, E.: Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science 277, 949-952 (1997).

Klaasen, H.L.B.M., Koopman, J.P., Poelma, F.G.J., and Beynen, A.C.: Intestinal, segmented, filamentous bacteria. FEMS Microbiol. Rev. 88, 165-180 (1992).

Klaasen, H.L.B.M., Van der Heijden, P.J., Stok, W., Poelma, F.G.J., Koopman, J.P., Van den Brink, M.E., Bakker, M.H., Eling, W.M.C., and Beynen, A.C.: Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. Infect Immun 61, 303-306 (1993).

Marco, A.J., Prats, N., Ramos, J.A., Briones, V., Blanco, M., Dominguez, L., and Domingo, M.: A microbiological, histopathological and immunohistological study of the intragastric inoculation of *Listeria monocytogenes* in mice. J. Comp. Pathol. 107, 1-9 (1992).

Marco, A.J., Altimira, J., Prats, N., López, S., Dominguez, L., Domingo, M, and Briones, V.: Penetration of *Listeria monocytogenes* in mice infected by the oral route. Microb. Pathogen. 23, 255-263 (1997).

Marra, A., and Isberg, R.R.: Invasin-dependent and invasin-independent pathways for translocation of *Yersinia pseudotuberculosis* across the Peyer’s patch intestinal epithelium. Infect. Immun. 65, 3412-3421 (1997).

Meynell, H.M., Thomas, N.W., James, P.S., Holland, J., Taussig, M.J., and Nicoletti, C.: Up-regulation of microsphere transport across the follicle-associated epithelium of Peyer's patch by exposure to *Streptococcus pneumoniae* R36a. FASEB J. 13, 611-619 (1999).

Neutra, M.R., Frey, A., and Kraehenbuhl, J.P.: Epithelial M cells: gateways for mucosal infection and immunization. Cell. 86, 345-348 (1996).

Neutra, M.R., Mantis, N.J., Frey, A., and Giannasca, P.J.: The composition and function of M cell apical membranes: Implications for microbial pathogenesis. Semin. Immunol. 11, 171-181 (1999).

Niedergang, F., and Kraehenbuhl, J.P.: Much ado about M cells. Trends Cell Biol. 10, 137-141 (2000).

Norris, T.L., Kingsley, R.A., and Bäumler, A.J.: Expression and transcriptional control of the *Salmonella typhimurium lpf* fimbrial operon by phase variation. Mol. Microbiol. 29, 311-320 (1998).

Owen, R.L.: M cells as portals of entry for HIV. Pathobiology 66, 141-144 (1998a).

Owen, R.L.: Mid-life crisis for M cells. Gut 42, 11-12 (1998b).

Pascopella, L., Raupach, B., Ghori, N., Monack, D., Falkow, S., and Small, P.L.C.: Host restriction phenotypes of *Salmonella typhi* and *Salmonella gallinarum*. Infect. Immun. 63, 4329-4335 (1995).

Penheiter, K.L., Mathur, N., Giles, D., Fahlen, T., and Jones, B.D.: Non-invasive *Salmonella typhimurium* mutants are avirulent because of an inability to enter and destroy M cells of ileal Peyer's patches. Mol. Microbiol. 24, 697-709 (1997).

Perdomo, O.J.J., Cavaillon, J.M., Huerre, M., Ohayon, H., Gounon, P., and Sansonetti, P.J.: Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. J. Exp. Med. 180, 1307-1319 (1994).

Phillips, A.D., Navabpour, S., Hicks, S., Dougan, G., Wallis, T., and Frankel, G.: Enterohaemorrhagic Escherichia coli O157:H7 target Peyer’s patches in man and cause attaching-effacing lesions in both human and bovine intestine. Gut 47, 377-381 (2000).

Phillips, A.D., and Frankel, G.: Intimin-mediated tissue specificity in Enteropathogenic Escherichia coli interaction with human intestinal organ cultures. J. Infect. Dis. 181, 1496-1500 (2000).

Pron, B., Boumaila, C., Jaubert, F., Sarnacki, S., Monnet, J-P., Berche, P., and Gaillard, J-L.: Comprehensive study of the intestinal stage of listeriosis in a rat ligated ileal loop system. Infect. Immun. 66, 747-755 (1998).

Sansonetti, P.J., and Phalipon, A.: M cells as ports of entry for enteroinvasive pathogens: Mechanisms of interaction, consequences for the disease process. Semin. Immunol. 11, 193-203 (1999).

Sansonetti, P.J., Arondel, J., Cantey, J.R., Prévost, M-C., and Huerre, M.: Infection of rabbit Peyer’s patches by *Shigella flexneri*: Effect of adhesive or invasive bacterial phenotypes on follicle-associated epithelium. Infect. Immun. 64, 2752-2764 (1996).

Savidge, T.C., Smith, M.W., James, P.S., and Aldred, P.: *Salmonella*-induced M-cell formation in germ-free mouse Peyer's patch tissue. Am. J. Pathol. 139, 177-184 (1991).

Savidge, T.C.: The life and times of an intesti-

nal M cell. Trends Microbiol. 4, 301-306 (1996).
Schulte, R., Kernéis, S., Klinke, S., Bartels, H., Preger, S., Kraehenbuhl, J-P., Pringault, E., and Autenrieth I.B.: Translocation of *Yersinia enterocolitica* across reconstituted intestinal epithelial monolayers is triggered by *Yersinia* invasin binding to β1 integrins apically expressed on M-like cells. Cellular Microbiol. 2, 173-185 (2000).
Sharma, R., van Damme, E.J.M., Peumans, W.J., Sarsfield, P., and Schumacher, U.: Lectin binding reveals divergent carbohydrate expression in human and mouse Peyer's patches. Histochem. Cell Biol. 105, 459-465 (1996).
Siebers, A., and Finlay, B.B.: M cells and the pathogenesis of mucosal and systemic infections. Trends Microbiol. 4, 22-29 (1996).
Takeuchi, A.: Electron microscope studies of experimental Salmonella infection. I. Penetration into the intestinal epithelium by *Salmonella typhimurium*. Am. J. Pathol. 50,109-136 (1967).
Takeuchi, T., Kitagawa, H., Imagawa, T., and Uehara, M.: Apoptosis of villous epithelial cells and follicle-associated epithelial cells in chicken cecum. J. Vet. Med. Sci. 61, 149-154 (1999).
van der Velden, A.W.M., Bäumler, A.J., Tsolis, R.M., and Heffron, F.: Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. Infect Immun. 66, 2803-2808 (1998).
Vazquez-Torres, A., Jones-Carson, J., Bäumler, A.J., Falkow, S., Valdivia, R., Brown, W., Le, M., Berggren, R., Parks, W.T., and Fang, F.C.: Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. Nature 401, 804-808 (1999).
Vazquez-Torres, A., and Fang, F.C.: Cellular routes of invasion by enteropathogens. Curr. Opinion Microbiol. 3, 54-59 (2000).
von Moll, L.K., and Cantey, J.R.: Peyer's patch adherence of enteropathogenic *Escherichia coli* strains in rabbits. Infect. Immun. 65, 3788-3793 (1997).
Wallis, T.S., Starkey, W.G., Stephen, J., Haddon, S.J., Osborne, M.P., and Candy, D.C.A.: The nature and role of mucosal damage in relation to *Salmonella typhimurium*-induced fluid secretion in the rabbit ileum. J. Med. Microbiol. 22, 39-49 (1986).
Wassef, J.S., Keren, D.F., and Mailloux, J.L.: Role of M cells in initial antigen uptake and in ulcer formation in the rabbit intestinal loop model of shigellosis. Infect. Immun. 57, 858-863 (1989).
Wilson, M., Seymour, R., and Henderson, B.: Bacterial perturbation of cytokine networks. Infect. Immun. 66, 2401-2409 (1998).