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M cell-targeted delivery of vaccines and therapeutics

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Background: M (microfold or membranous) cells are specialised epithelial cells responsible for antigen sampling at the interface of mucosal surfaces and the environment. Their high transcytotic ability make M cells an attractive target for mucosally delivered vaccines and therapeutics. *Objective*: This brief review discusses the current state of M cell-targeted mucosal delivery systems and the potential of such delivery systems for the development of new vaccines and therapeutics against mucosal infectious and inflammatory diseases. *Scope*: A variety of synthetic microparticles/nanoparticles have been developed and tested as vehicles for M cell-targeted mucosal drug and vaccine delivery. β1 integrins, pathogen recognition receptors, specific carbohydrate residues and other M cell surface antigens have been exploited as potential targets for the delivery of mucosal vaccines and therapeutics. *Conclusion*: Despite a considerable body of literature, much work still needs to be done before an effective M cell-targeted vaccine or therapeutic is developed.

Keywords: M cells, mucosal immunity, targeted delivery, therapeutics, vaccine

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1. Introduction

Mucosally delivered drugs and vaccines have many advantages over their parenteral counterparts [1]. Mucosal diseases may well be better treated with direct, local delivery rather than systemic drug delivery to the affected mucosal surface, so that most or all of the drug administered reaches its target, rather than circulating through the entire body, decreasing the bioavailability of the drug at the mucosal target site. For example, it is well established that the use of inhalers to administer asthmatic therapeutics locally is superior to oral or parenteral administration in that much lower doses are needed and fewer adverse effects are seen [2]. Also, from a practical standpoint, mucosal drugs and vaccines are easy to administer and, unlike parenteral drugs/vaccines, do not require trained personnel to administer an injectable compound. Patient compliance is higher when there is no needle stick involved and costs are substantially lower when trained personnel are not required. From a global perspective, it also becomes much more feasible to launch large-scale vaccination and treatment strategies in less industrialised countries where cost and trained personnel are important considerations [3].

The delivery of vaccines by mucosal routes also has several additional benefits when compared to parenteral routes [1,4]. First, mucosal surfaces are the interface between the body and the environment and, as such, they are most likely to be the port of entry for foreign antigens. In fact, most infectious diseases are initiated at a mucosal surface. Not only can mucosal immunisation induce a strong mucosal immune response, which generally parenteral immunisation cannot, mucosal vaccine administration can also induce a systemic immune response. In addition, administration of a vaccine at a mucosal site can activate the common mucosal immune system, thereby inducing an immune response at

mucosal sites distal to the administration. Consequently there is potential that vaccine administration via one mucosal site can protect against infection at a distal mucosal site. For example, it has been shown that HIV vaccine administered intranasally to female macaques can induce strong antibody response in saliva, faeces and vaginal washings. More importantly, intranasal vaccine administration was more protective (three out of four macaques) than intramuscular vaccination (one out of four macaques) after intra-vaginal challenge with chimeric simian-HIV [5].

Despite the obvious advantages of mucosal vaccines, only a very few mucosal vaccines (< 10) have made it to market and even fewer are currently approved for use in the general population. The oral (Sabin) polio vaccine has been most effective to protect against polio, followed by the Ty21a vaccine strain of *Salmonella typhi*, which protects against typhoid when administered orally [6]. Other mucosally administered vaccines have some efficacy against disease caused by *Vibrio cholera*, rotavirus, tuberculosis and adenovirus (which is restricted to military personnel) [4]. An oral live-attenuated vaccine against rotavirus and a nasal enterotoxin-adjuvanted inactivated influenza vaccine were both brought to market but were later withdrawn due to serious side effects [1].

The small number of approved vaccines against mucosal infections is indicative of the difficulties associated with the development of an effective mucosal vaccine. To be effective, any mucosally delivered drug/vaccine must reach and breach the epithelial barrier. The mucosal epithelium is composed of a thin layer of cells sealed at their apical membranes by tight junctions. Mucus and secretory IgA further protect the epithelium and the thick glycocalyx of the cell wall contains hydrolytic enzymes that degrade most pathogens and macromolecules [7]. Orally delivered drugs/vaccines have the additional challenges of surviving the harsh gastric and intestinal environments and being present in high enough concentrations that they are not too diluted in the intralumenal fluid of the gut [8]. The majority of research on M (microfold or membranous) cell-targeted delivery of vaccines and therapeutics has focused on oral delivery of vaccine candidates and, as such, this will be the focus for most of this brief review. However, many of the issues facing the development of oral vaccines are relevant for other routes of administration as well as for effective mucosal delivery of therapeutic agents.

2. Anatomy and function of the intestinal M cell

The mucosal surface of the gastrointestinal tract is constantly exposed to high levels of antigens which must be monitored for potentially harmful agents (such as pathogens) while discriminating these from harmless food and non-pathogenic antigens. The membranous or microfold cell (M cell) in the Peyer's patches is the primary cell type responsible for sampling foreign particulate antigens and presenting them to the intestinal immune system, which will mount either an immunological response or tolerance to the antigen. Due to its nature, the gut typically exhibits immune tolerance, which prevents the development of unwanted inflammatory responses and food allergies [9], but unfortunately this also impedes the development of oral vaccines [10].

M cells are specialised epithelial cells mainly found in the follicle-associated epithelium (FAE) overlying Peyer's patches and other lymphoid aggregates (**Figure 1**). These cells are distinguished from surrounding epithelial cells by the absence of the surface microvilli that are characteristic of intestinal epithelial cells. Instead, the apical membrane of the M cell has a microfold (or membranous) topography, hence the name M cell. Although M cells also form tight junctions with adjacent cells to maintain the epithelial barrier, they have a deeply invaginated basolateral membrane which contains infiltrating lymphocytes [11]. Consequently, once antigens are transcytosed by the M cell they can quickly move to the basolateral membrane to reach and interact with underlying lymphoid aggregates [12].

The M cell may be the preferred cell type for mucosally targeted drug/vaccine delivery, but it should be mentioned that other cell types at mucosal surfaces do have some antigen sampling ability. For instance, enterocytes have limited ability to transcytose antigens, but this may be compensated for given the much higher percentage of enterocytes in the intestine [13]. In addition, dendritic cells, the professional antigen-presenting cells, can directly sample antigens from mucosal surfaces [13]. However, the M cell is still most attractive for targeted drug/vaccine delivery because of its high transcytotic activity at the FAE. Although villous M cells located outside the FAE have also been identified [14], the M cells of the FAE remain the primary vehicles for antigen sampling and subsequent induction of mucosal immune responses [7]. As such, the FAE has lower amounts of secretory IgA and mucus and the M cells of the FAE have a much thinner surface glycocalyx as compared to other intestinal epithelial sites, allowing for easier antigen sampling [15,16].

Although M cells constitute 10 – 50% of cells of the FAE overlying Peyer's patches, overall, there is only approximately one M cell for every 10 million epithelial cells in the intestinal tract [17]. Historically, oral vaccines have simply bombarded the system with antigen in the hope that enough will persist to be transported across the epithelial barrier. This is, however, a very inefficient means of presenting antigen to M cells.

3. Targeting the M cell for vaccine/therapeutic delivery

Since the percentage of M cells in mucosal epithelium is very low, various means have been used to attempt to

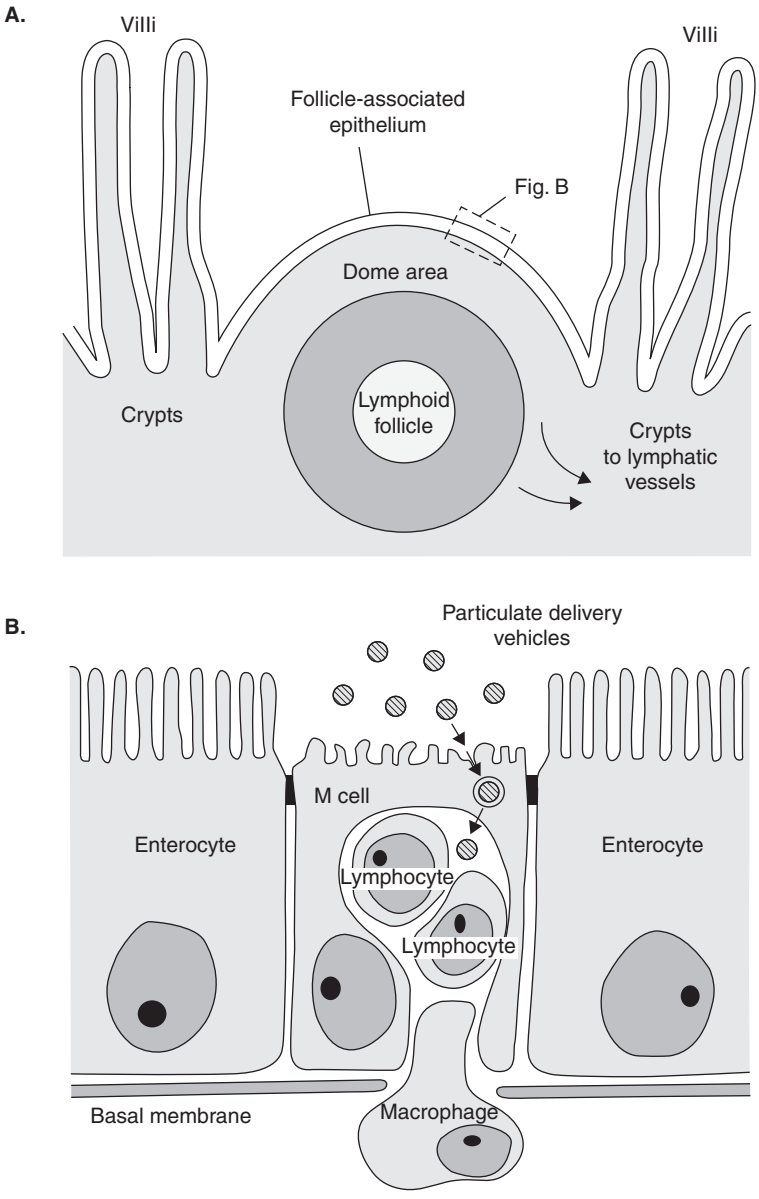

Figure 1. Schematic transverse sections of a Peyer's patch lymphoid follicle and overlying follicle-associated epithelium (FAE), depicting M cell transport of particulate delivery vehicles. The general structure of intestinal organised mucosa-associated lymphoid tissues (O-MALT) is represented by the schematic transverse section of a Peyer's patch lymphoid follicle and associated structures in (**A**). The lymphoid follicle is situated beneath a dome area which protrudes into the gut lumen between villi and which is covered by the FAE. This epithelium is characterised by the presence of specialised antigen sampling M cells (depicted in **B**). These cells typically possess a reduced number of irregular microvilli on their apical surface and a basolateral cytoplasmic invagination which creates a pocket harbouring lymphocytes and macrophages. Particulate delivery vehicles are largely prevented from passing between epithelial cells by tight junctions. However, since M cells possess a relatively high transcytotic capacity compared to that of enterocytes, the M cell portal may represent an efficient route for the transport of drugs and vaccines carried by particulate delivery vehicles across the intestinal epithelial barrier. Synthetic delivery vehicles may be targeted to M cells by coating with appropriate ligands such as lectins or microbial adhesions, or the delivery vehicle may consist of a live attenuated microorganism which innately targets to M cells. After adherence to the M cell apical membranes and transport across the thin apical cytoplasmic rim, reagents are delivered to the underlying inductive O-MALT sites and may subsequently disseminate via the lymphatics. Reprinted from Clark MA, Jepson MA, Hirst BH. Exploiting M cells for drug and vaccine delivery. Adv Drug Deliv Rev 2001;50:81-106. Copyright (2001), with permission from Elsevier.

increase interaction of putative vaccines and therapeutics with M cells. Previously, oral vaccine formulations have been administered at extremely high doses to allow for enough vaccine particles to survive the harsh gastric environment and have sufficiently concentrated vaccine present at the intestinal epithelium to be sampled by the very limited number of M cells. An alternative way to improve M cell interaction would be to increase the actual number of M cells available for antigen sampling. In fact, it has been shown that exposure to *Streptococcus pneumoniae* R36a induces a rapid and significant increase in the number of fully functioning M cells available for antigen sampling [18,19]. The danger here is that higher M cell numbers would translate into more sampling of all antigens, not just those of interest. This would lead to increased risk for food allergies and inflammatory diseases [17]. Instead, perhaps the most logical and promising way to increase interaction of antigens of interest with M cells is to specifically target putative vaccines and therapeutics to the apical surface of M cells.

4. Live attenuated vaccine strains and live vectors

The major function of the M cell is to serve as a surveillance mechanism for potential pathogens. However, some micro-organisms are able to exploit this function and use it as a means to gain access to underlying mucosal tissue. For instance, *S. typhimurium* targets murine M cells via long polar fimbriae [20] and its Type 3 secretion system also plays a role in uptake by M cells, while *Yersinia* uses invasins to bind to cell surface β1 integrins, which are mainly found on the basolateral membranes of polarised epithelia, but are also expressed on the apical surface of M cells. In fact, M cells can be differentiated from surrounding enterocytes by the surface expression of β1 integrins [21]. As such, it is a logical step to try to use live attenuated strains of these bacteria as vaccines themselves or delivery vehicles by which heterologous antigens can be presented to the mucosal immune system. The most well-known mucosal vaccines are the oral polio vaccine, which uses three live attenuated poliomyelitis virus strains [6], and the Ty21a vaccine strain of *S. typhi*, which protects against typhoid [22]. More recently, researchers have tried to take advantage of the natural M cell-targeting ability of *Salmonella* and other bacteria and have used them to express heterologous antigens and serve as live vectors which act as delivery vehicles to present antigens of interest to M cells. Promising research includes work on shigellosis [23], *Helicobacter pylori* [24], anthrax [25] and HIV [26].

However, there are regulatory concerns regarding the use of live attenuated bacteria as vaccines or live vectors, as there is always the risk of reversion to a fully active bacterium and as such they are generally considered unsafe for use in humans [8]. Since the main function of the M cell is to bind and uptake foreign particulate antigens for presentation to underlying immune cells, it is not only able to transcytose microbial antigens, but also other particulates, including synthetic microparticles. If a synthetic delivery vehicle could be used to target the M cell, then the problems associated with the use of a live vector would be circumvented.

5. Synthetic delivery vehicles

A wide variety of synthetic particles have been shown to interact with M cells, including poly (DL-lactide-co-glycolide) (PLG) microparticles, polystyrene (latex) microparticles and liposomes [27]. However, not all particles bind equally to M cells. A series of experiments by Frey *et al.* (1996) used 1.13 μm latex particles as well as 28.8 nm gold particles all coated with the B subunit of cholera toxin (CTB) and showed that the nanometer-sized gold particles were able to bind to M cells but not to enterocytes of rabbit Peyer's patches, while the larger micron-sized latex particles did not bind to either cell [15]. They suggested that the thinner glycocalyx of the M cell was responsible for the preventing the larger particles from binding to the M cell. Other studies have compared the uptake of various sizes of latex particles ranging from 50 nm to 3 μm and found that uptake by rat Peyer's patches both after oral administration [28] and in a rat intestinal loop model [29] increased as particle size decreased. Hydrophobicity and surface charge of the delivery vehicle are other parameters that can influence the ability of the particle to reach and interact with M cells. However, negatively charged mucus may neutralise positively charged particles and surface hydrophobicity may go unnoticed since proteins in the gut lumen may hide hydrophobic regions [27]. Vyas and Gupta (2007) have recently discussed in great detail a variety of factors affecting the delivery of nanoparticles/microparticles including particle size, hydrophobicity, surface charge, dose, species of animal used, method of quantification of uptake, etc [1]. These factors have led to a significant amount of inconsistency in the experimental outcome between different model systems.

Despite the technical complexities in the development of an M cell-targeted delivery system, there remains strong interest in the development of synthetic vehicles for use in mucosal drug and vaccine delivery. Intrinsic adjuvant activity would be advantageous to any delivery vehicle and, in fact, a number of particulate delivery systems, including PLG micro and nanoparticles, liposomes and CT have the ability to stimulate both the cell-mediated and humoral arms of the immune system [30]. The use of PLG micro-particles in vaccines and therapeutics is particularly attractive because these microparticles are made of a biodegradable polymer that can protect the compound from the harsh gastric/intestinal environment during delivery, but then gradually degrades to allow for slower, more sustained release of the drug/vaccine. The ratio of lactic and glycolic acids [27], as well as the chemistry of the terminal ends

of the polymer [31], determine the rate of degradation and can be adjusted to suit the requirements of drug delivery. Liposomes also have some promise as potential delivery vehicles for mucosal vaccines and therapeutics since they can encapsulate both lipid soluble and aqueous agents [32]. Thus, subunit or DNA vaccines with lower immunogenicity than live attenuated vaccines can increase their potency by encapsulation in liposomes or coating of synthetic delivery particles. However, limited data from various experimental model systems suggests that simply coating or encapsulating the particles with the antigen of interest is unlikely to be very effective. Instead, conjugation of the particles to ligands that specifically bind the apical surface of M cells is far more likely to yield positive results [3].

6. Targeting the apical membrane of the M cell

Information about M cell-specific surface carbohydrates and receptors is sparse. What is known has been, for the most part, determined from studies examining the mechanisms by which microbial pathogens are able to invade the M cell. Just as pathogens can exploit the M cell to gain access to the underlying tissue, drug delivery can be targeted to the M cell by using the same strategies that pathogens use.

6.1 β1 integrins

Invasins from the *Yersinia* species are responsible for binding to β1 integrins expressed on the apical membrane of M cells in mouse Peyer's patches [21] and in an *in vitro* Caco-2/Peyer's patch co-culture model of M cells [33]. Researchers have exploited this and coated invasin-C192 (purified invasin) on latex nanoparticles, which were then administered orally to rats [34]. Six times more invasin-coated nanoparticles were found in the systemic circulation than uncoated nanoparticles and histological examination showed strong binding of invasin-C192 nanoparticles in rat ileum [34].

The *in vitro* M cell model itself highlights the importance of β1 integrin expression on M cells. Redistribution of α5β1 integrin from the basolateral membrane to apical membrane of cells in a Caco-2/Peyer's patch co-culture model is a hallmark of the transformation process of Caco-2 cells (enterocyte-like cells) to M-like cells [35] and this is associated with an increase in transcytosis of killed non-typeable *Hemophilus influenzae*, which is inhibited by antibodies to α5β1 integrin [17]. Therefore it was suggested that targeted α5β1 administration may increase the number of M cells in the FAE; however, this would be a double-edged sword. More M cells would mean more targets for the vaccine formulation, but also more targets for any antigen passing through the gut lumen. Thus, there is an increased risk of the development of food allergies and inflammatory disease.

Fibronectin is the endogenous ligand for α5β1 integrin, and its RGD peptide motif (Cys-Gly-Arg-Gly-Asp-Ser-Tyr) has recently been investigated as a potential ligand to target M cells. Gullberg *et al.* (2006) observed significantly higher binding of nanoparticles to β1 integrins of human Peyer's patches and increased transport across the epithelium when Arg-Gly-Asp (RGD) was coated on nanoparticles in order to target β1 integrins [36]. A subsequent study using RDG-coated PLG nanoparticles has showed its potential to induce ovalbumin-specific antibodies after oral immunisation [37].

6.2 Pathogen recognition receptors (PRRs)

Immunofluorescence studies in an *in vitro* M cell model and in isolated intestinal tissue segments have shown that M cells express pathogen recognition receptors (PRRs) such as platelet activating factor receptor (PAFR) and toll-like receptor (TLR)-4, but not TLR-2 [35]. Subsequently, Tyrer *et al.* (2007) used killed non-typeable *H. influenzae* and showed that anti-TLR-2 had no effect on the number of bacteria transcytosed in an *in vitro* M cell model, while both anti-TLR-4 and anti-PAFR significantly decreased the number of bacteria transcytosed. The same pattern was observed in murine Peyer's patches *in vivo*. Potentially, the presence of these PRRs could be exploited by delivering LPS, CpG DNA or other pathogen-associated molecular patterns (PAMPs), along with vaccine candidates [17].

6.3 Immunoglobulin and monoclonal antibody-mediated targeting

IgG and IgA can both adhere selectively to the M cell apical membrane and, when coated with latex microparticles, increase both binding and uptake by M cells in an *ex vivo* model [38]. Also, rectal immunisation of mice with CTB-adjuvanted ferritin-encapsulated liposomes coated with IgA induces enhanced local ferritin-specific IgA as compared to those immunised with non-coated liposomes [39]. This effect was thought to be mediated by a novel IgA receptor that has been found on mouse M cells [40].

Monoclonal antibodies (Mab) against M cell surface antigens may be another way to target the apical surface of M cells. In fact, an antirabbit Mab specific to FAE M cells was coated on latex microspheres was able to bind to and be transcytosed by M cells in a rabbit intestinal loop model [41]. Recently, another Mab, NKM 16-2-4, was reported as an novel means to target vaccine antigens to M cells [42]. Specifically, this Mab recognises the α(1,2)-fucose moiety on M cells both in the FAE as well as the more recently discovered villous M cells without reacting with either epithelial or goblet cells. More importantly, when administered orally as a conjugate to botulinum toxoid together with CT, NKM 16-2-4 was able to elicit strong serum and mucosal antibody responses and protected mice against lethal challenge with botulinum toxin, while a control rat IgG conjugate vaccine preparation was not protective [42].

6.4 Lectins

Carbohydrates on the thin glycocalyx of the FAE are differentially expressed from those on the thicker glycocalyx

of surrounding enterocytes [43,44]. Specifically, lectins recognising specific carbohydrate residues were used to differentiate the various microcompartments of the intestinal epithelium. As a result, the specific lectin-binding characteristics of M cells have over the years been the subject of a good deal of research aimed at M cell-targeted delivery. Coincidentally, *S. typhimurium, Y. enterocolitica* and *E. coli* RDEC-1 all have lectin and all utilise M cells to penetrate the epithelium [45]. *Ulex europaeus* agglutinin-1 (UEA-1) is probably the most promising candidate lectin as it binds almost exclusively to the apical surface of M cells in fresh or fixed murine Peyer's patch tissue, as well as ligated intestinal loops [46,47]. When administered orally or injected into ligated intestinal loops of anesthetised mice, UEA-1 coated latex microparticles selectively targeted and were rapidly endocytosed by mouse Peyer's patches [48]. The potential use of lectins, and UEA-1 in particular as a component in oral vaccines was proposed by Chen, Torchilin and Langer (1996), who found that UEA-1 coating of polymerised liposomes increase uptake and distribution of liposomes [49]. This may well be due to targeting of the M cell since, in an intestinal loop model, polymerised liposomes were able to target M cells when coated with UEA-1 [50]. Recently, hepatitis B surface antigen (HBsAg) was loaded on microparticles coated with UEA-1 to target murine M cells. Oral vaccination yielded comparable serum-specific anti-HBsAg antibodies to intramuscular vaccines with alum-HBsAg; however, oral immunisation was also able to elicit sIgA production in intestinal, vaginal and salivary secretions [51]. Lectin-mediated M cell-targeting has also been explored for use in intranasal administration. This is discussed in a later section. Unfortunately, in humans, UEA-1 binds strongly to enterocytes with little or no binding to M cells [52]. Although a lectin that binds human M cells has not been identified so far, perhaps lectin mimetics can be developed to specifically target human M cells [53]. Regardless, lectin-mediated M cell target is still a valid experimental model to help understand the basic mechanisms of M cell-targeted drug/vaccine delivery.

6.5 Potential human M cell targets

It was initially reported that human M cells display sialyl Lewis A antigen [52] which differs from the Lewis A antigen on the surface of epithelial cells by a single $\alpha(2-3)$ sialic acid. Antibodies to sialyl Lewis A antigen bound about 80% of M cells but only about 20% of enterocytes, raising the possibility to explore the Lewis A antigen as a potential target for mucosal drug and vaccine delivery. However, the preferred expression of sialyl Lewis A antigen by human M cells phenomenon has not yet been reproduced by other researchers [54]. More recently, high levels of expression of β1-integrin and CD9 were observed in both an *in vitro* M cell model and in the FAE of human Peyer's patches [36]. Also, microarray analysis of a Caco-2 co-culture showed upregulation of Galectin 9, which was confirmed by immunohistochemistry in both the *in vitro* M cell model and human tissue sections [55]. These newly identified surface markers provide additional targets for potential delivery of mucosal vaccine or therapeutics through the M cell and for the improvement of the efficacy of the vaccine and therapeutic formulations.

7. Respiratory delivery of mucosal vaccines and therapeutics

The majority of research on M cell-targeted vaccine and therapeutic delivery has so far focused on the intestinal tract; however, it is worth noting that the respiratory tract is a much less harsh environment that also contains less fluid and as such the dilution factor would be far lower [56]. Moreover, M cells are present in the nasal associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) of several animal species including rabbit, rat and mouse [27]. Results from limited studies on the M cell in the upper respiratory tract have corroborated findings from studies with intestinal M cells. For example, liposomes and polystyrene microparticles are transcytosed across the upper respiratory tract epithelium [57] and lectin binds preferentially to M cells of the upper respiratory tract of rats [58]. Also, intranasal immunisation of hamsters with M cell-selective lectin (GS I-B4) conjugated to horseradish peroxidase (HRP) induced a stronger serum IgG response than GS I-B4 admixed with HRP or HRP alone [59]. The feasibility of delivering M cell-targeted vaccines via the respiratory tract was demonstrated when Wu *et al.* (2001) conjugated polylysine with reovirus protein ς1, an M cell ligand that binds the apical surface of M cells in NALT, and used this conjugate as a vehicle for the mucosal delivery of DNA vaccines [60]. Intranasal immunisation with the protein ς1-polylysine-DNA complexes not only induced antigen-specific serum IgG and mucosal IgA responses, but also enhanced cell-mediated immunity including elevated cytotoxic T lymphocyte responses in the lung. More recently, Manocha *et al.* (2005) showed that HIV peptides entrapped in UEA-1-coated PLG elicit an enhanced systemic and, more importantly, mucosal immune response in mice after intranasal administration [61]. These findings are encouraging; however, although it is widely believed that there are M cells in the human respiratory tract, their presence has not yet been unequivocally demonstrated.

8. M cell-targeted immunotherapy for induction of mucosal tolerance

Mucosal tolerance is an essential mechanism which allows for the body to come into contact with harmless foreign antigens such as food and commensal bacteria without mounting an immunological response that is potentially harmful to the host. The induction of appropriate mucosal tolerance can, therefore, be a means to treat inflammatory diseases such as inflammatory bowel disease (Crohn's disease

and ulcerative colitis), autoimmune disorders and allergy. Although the major thrust of research on targeting M cells for vaccine and therapeutic delivery has so far focused on enhancing the vaccine immunogenicity and drug efficacy, researchers have now started to explore the potential of M cell-targeted vaccines and immunotherapies for induction of immune tolerance or immunosuppression as a means to treat inflammatory and autoimmune diseases. For instance, poly(DL-lactic acid), which is taken up by M cells [62], was used to encapsulate dexamethasone. Oral administration of dexamethasone-containing microspheres was shown to be a more effective treatment in a mouse model of colitis than dexamethasone alone [63]. The potential for exploiting M cells to treat inflammatory disease was also examined by Roth-Walter *et al.* (2005), who again used poly(DL-lactic acid) microspheres, this time coated with lectin and loaded with birch pollen allergens. After an oral gavage regimen, birch-pollen specific IgG2a but not IgG1 was detected. It was suggested that these results showing a Th1-antibody response may counterbalance the Th-2-dominated allergy response [64]. Research into the mucosal delivery of immunotherapeutics for the treatment of inflammatory disease is still in its infancy, but it is a field that has already yielded interesting results.

9. Expert opinion

There is currently an unmet demand for the development of effective M cell-targeted delivery systems for mucosal vaccine and therapeutic delivery. Such systems are essential for successful combat against infectious diseases (such as HIV, hepatitis B and tuberculosis) and inflammatory and autoimmune diseases (such as IBD and asthma). Over the last few decades, substantial research efforts have been invested in understanding the fundamental biology of M cells and the exploitation of their use in targeted mucosal vaccine and therapeutic delivery. One of the initial major stumbling blocks was the lack of a suitable M cell model. Since the percentage of M cells in the mucosal surface is very low, the use of *in vivo* models is logistically difficult. Hence the development and subsequent refinement of the current Caco-2 coculture *in vitro* M cell model [33,65] has provided a relatively reliable and reproducible research tool for the study of M cells. This model has since played an important role in furthering our knowledge of the role of M cells in antigen sampling, translocation and induction of mucosal immune responses, as well as providing an alternative method to study potential M cell-targeted vaccines and therapeutics. For instance, its use in conjunction with gene expression profiling techniques may help yield new M cell targets for mucosal drug and vaccine delivery over the next few years.

Although many advances have been made in targeting M cells for mucosal vaccine and therapeutic delivery, the precise significance of M cells in the induction of mucosal immunity/tolerance remains to be determined and confirmed. Consequently, the vast research and development efforts into M cell-targeted vaccine and therapeutic delivery over the past decades has failed to transform the great potential and expectations of this technology into clinical realities. The frustrations and disappointments encountered in the utilisation of the M cell as a target for vaccine and therapeutic delivery are probably partially due to the relative paucity of M cells in mucosal surfaces, which has not only confounded the confirmation of an essential role for M cells in mucosal immunity, but has also cast doubt on their actual *in vivo* significance. In this regard, the majority of research work into the significance of M cells in mucosal immunity was performed using large and unnatural amounts of reagents in either the *in vitro* co-culture M cell model (up to a 500:1 ratio of bacteria to cells) [33] or in a ligated intestinal loop system (up to 4×10^9 bacterial cells per ligated intestinal loop) [66]. In addition, only a proportion of cells in the *in vitro* co-culture system share the key features of M cells [65]. Consequently, the system suffers from variations in the proportion of M cells between experiments and laboratories, which has undoubtedly contributed to a significant amount of inconsistency between laboratories. Even with *in vivo* immunisation studies performed in laboratory animals [23,37], it is often difficult to convincingly demonstrate that the M cell is the sole cell type responsible for the observed biological effect. However, recent advances in the development of transgenic or chimeric animal models with a complete disruption of the ontogeny of M cells are likely to provide definitive answers about the function of M cells in mucosal immunity in the future.

More basic fundamental research is still needed to better understand how M cells sample antigens and transcytose them to the basolateral membrane. Also, there are some basic technical considerations for mucosal delivery via M cells. The development of new approaches to transitionally or conditionally enhance the number and function of M cells is likely to reduce the concerns about the potential induction of unwanted mucosal inflammation associated with M cell targeting. In addition, since most microparticles administered orally become trapped in the mucus and only a small fraction of them enter mucosal inductive sites [8], intranasal administration is a viable alternative, since there is less mucus at this site. Although the intranasal route for delivery of mucosal vaccines and therapeutics has been exploited by many researchers during the last decade, the presence of M cells in human NALT and BALT still has not yet been positively confirmed. Nevertheless, administration of vaccines via the respiratory route (such as aerosol and nasal spray) has been used successfully in human immunisation against measles and influenza, although the role of M cells in these cases is not clear.

The current repertoire of M cell targets is still extremely small. In particular, very little is known about cell surface receptors that are specific to human M cells. We foresee that

significant effort will be made in the next few years to identify novel M cell targets. The use of the *in vitro* model of human M cells, despite its drawbacks, in conjunction with microarray analysis and other gene profiling techniques should yield more human M cell targets that can be exploited for the purpose of developing effective mucosal vaccines and therapeutics. In addition, much of the earlier synthetic particle delivery studies used microparticles or very large nanoparticles. There are many factors that affect uptake of a delivery vehicle, but it is known that M cells will only uptake small particulate antigens. Thus, some of the previous conflicting results obtained using larger-sized particles may warrant repeating with smaller-sized particles that more effectively target M cells.

The next few years should also see more exploratory studies on targeting M cells to induce mucosal tolerance for the prevention and treatment of autoimmune diseases, chronic inflammatory diseases and food and environmental allergies. In this regard, TGF-β in breast milk seems to have some involvement in the anti-allergy properties of human milk and when TGF-β is given orally to mice, it induces tolerance to ovalbumin antigen [67]. An effective M cell-targeted delivery of TGF-β-coated nanoparticles to infants may prevent the development of food and environmental allergies that sometimes occur in formula-fed children. Also, since some mucosal tolerance studies used CTB as an adjuvant, it would be interesting to examine the possible mucosal tolerogenic effect of coating nanoparticles with CTB. Thus a lot of work remains to be done. A deeper understanding of M cell function and apical cell surface antigens and receptors will be critical to the development of M cell-targeted vaccines and therapeutics.

Declaration of interest

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