Review

Unsolved mysteries of intestinal M cells

Summary
M cells are highly specialised cells present within the epithelium overlying organised lymphoid follicles of the small and large intestine. They play a central role in the initiation of mucosal immune responses by transporting antigens and microorganisms to the underlying lymphoid tissue. In this way the mucosal immune system encounters the limitless variety of antigens that enter the body through the gut mucosa and reacts by mounting specific mucosal and systemic immune responses.

Despite the role of M cells in mucosal defence many basic aspects of their biology, the most controversial being their origin within the follicle associated epithelium (FAE), still remain the subjects of debate. Recently, new information on the complex interactions of luminal microorganisms, mucosal immune system, and epithelial cells, that are instrumental in the induction of this cell phenotype, have become available. Here, the most novel data and hypotheses on M cell genesis and function in the gut are reviewed and discussed.

Introduction
The main task of the epithelium overlying mucosal surfaces of the intestinal tract is to provide an effective barrier to the vast majority of macromolecules and microorganisms present in the intestinal lumen. This is achieved by several means. Firstly, the epithelium is formed by cells joined by tight junctions that allow passage of water and ions but provide an effective mechanical barrier to macromolecules. Secondly, mucosal surfaces are covered by local secretions of mucus, secretory IgA antibodies, and by a thick glycolaxial. These features and the closely packed carpet of microvilli present on absorptive cells prevent contact and binding of macromolecules and potential pathogens to the epithelium. On the other hand, the intestinal epithelium must also provide portals through which antigens and microorganisms are delivered to the intestinal immune system in order to induce immune responses. In fact, it is now established that antigenic penetration of epithelial barriers is the first critical step in the generation of protective mucosal and systemic immune responses. The ability of the intestinal epithelium to transport antigens and microorganisms is strategical restricted to the FAE that overlies the organised mucosal associated lymphoid tissue in the gut. To accomplish this task, the FAE has evolved features that distinguish it from the surrounding absorptive epithelium. The most remarkable adaptation is the presence of a relatively small number of highly specialised antigen sampling membraneous (M) cells.

Features of intestinal M cells
MORPHOLOGY AND FUNCTION
M cells were first observed by transmission electron microscopy in rabbit appendix. They display distinctive morphological features that distinguish them from surrounding enterocytes. The brush border is poorly organised with short irregular microvilli, and the thick glycolaxial usually associated with absorptive cells is absent. These adaptations allow material in the intestinal lumen to have easy access to the apical domain of M cells where it is internalised and then transported to the underlying lymphoid tissue. The mechanisms by which M cells take up microorganisms and macromolecules vary according to the nature of this material. Large particles and bacteria induce phagocytosis, which is associated with ruffling of the apical plasma membrane of the M cell and rearrangement of the actin cytoskeleton, which permits active formation of pseudopod-like structures. Viruses and other adherent particles are taken up by endocytosis via clathrin coated vesicles, whereas non-adherent material is internalised by fluid phase endocytosis. In any case, internalisation is quickly followed by transport of endocytotic vesicles to the endosomal compartment and then by exocytosis to the basolateral membrane. The biochemical events involved in the intracellular transport of endocytotic vesicles in M cells have not been thoroughly explored but it appears that this is regulated in the same manner as polarised transport observed in other epithelial cells.

A typical feature of M cells is that, unlike other intestinal epithelial cells, the basolateral surface is deeply invaginated to form intraepithelial pockets that are in intimate contact with specialised lymphocytes that migrate to this peculiar compartment from lymphoid tissue (fig 1A, B). This modification of the basolateral domain of M cells is believed to be a way of shortening the distance that endocytotic vesicles have to travel to reach immunocompetent areas. Thus M cell pockets provide the first opportunity for contact between antigens, penetrating the epithelial barrier and specialised immune cells. It has been proposed that M cell pockets are the sites where intraepithelial lymphocytes (IEL) can interact early with internalised antigens in an environment sequestered from regulatory elements of the mucosal immune system.

SIMPLY ANTIGEN SAMPLING CELLS OR SOMETHING MORE?
The phenotype of IEL residing in M cell pockets has been analysed in Peyer’s patch tissue of humans and other species. Although there is remarkable variation between animal species, most of the M cell associated IEL are α/β memory T cells. In the rabbit a distinctive phenotype lacking both CD4 and CD8 was also observed. T cells in the pockets are often associated with naive IgM+ and IgD+ B cells and macrophages, whereas IgG and IgA+ cells have rarely been observed.

The lymphoid cells harboured within M cell pockets represent all the cell types required to initiate a specific immune response but their true functional significance remains unclear. An immunological role for the M cell associated lymphocytes is suggested by the observation that their number rapidly increased after application of a bacterial challenge. In these circumstances, a large number of cells migrate into the intestinal lumen via M cell gated diapedesis. The physiological relevance of M cell regulated passage of lymphocytes into the gut lumen and their role in this location remain to be determined.

A broadly accepted view is that M cells are simply conduits through which antigens reach the immunocompetent areas of the gut associated lymphoid tissue. According to some authors M cells are provided with the cytoplasmic

Abbreviations: FAE, follicle associated epithelium; IEL, intraepithelial lymphocytes.
components required to process antigens, including cathepsin E, an aspartic proteinase involved in antigen processing by a B cell lymphoma. It is notable that by several authors on the basis of the extreme variability in immune responses by signalling to lymphocytes by releasing potential to participate actively in the generation of mucosal facing the pockets. M cells may also have the potential expression of major histocompatibility complex class II contact with cells of the immune system. Taken together deeply protrude into the lymphoid tissue where they make contact with cells of the immune system. Enterobacteria (eb) processes that deeply protrude into the lymphoid tissue (asterisks) where they make contact with cells of the immune system. Enterobacteria (eb) and other antigens adhere to the apical area of the M cells and are subsequently internalised and transported to the mucosal immune system. M cells are also a migration route for lymphocytes moving into the intestinal lumen. The function of these intraluminal cells, the number of which markedly increases after bacterial challenge, remains unknown.

HETEROGENEITY OF M CELL POPULATION

M cells were discovered more than 25 years ago but despite intensive investigations a clear cut M cell marker, independent of species and location in the gut, has not been found. For example, in the rabbit, but not in other species so far tested, M cells express the intermediate filament protein vimentin that is typical of cells of mesenchymal origin. In the past, M cells have been mainly identified by the absence of hydrolytic enzymes, such as alkaline phosphatase, that are abundant in the brush border of enterocytes. However, the reliability of alkaline phosphatase as a specific negative marker has been questioned by several authors on the basis of the extreme variability in the content of this enzyme in all FAE cells. More recently it has been reported that mouse M cells display a different distribution of the actin associated protein villin. This is restricted to the apical region of enterocytes but in M cells it is diffusely distributed in the cytoplasm reflecting a differentially organised cytoskeleton and probably a decreased structural rigidity.

The heterogeneity of M cell populations is also well exemplified by the highly differentiated expression of polysaccharides of membrane bound glycoproteins and glycolipids. The use of a large panel of lectins to stain FAE cells has revealed that in some cases M cells display a different glycosylation state compared with neighbouring enterocytes. In mice, for example, small intestine Peyer’s patch M cells predominantly express α-(1–2)-fucose that can be detected by Ulex europaeus (UEA-1) lectin. The pattern is different in caecal patches where M cells are stained by the same lectin but also express other terminal saccharides. In humans, the glycosylation pattern is different from that of other species as M cells preferentially display the sialyl Lewis A antigen.

Membrane bound glycoconjugates are believed to play an important role in microbial-M cell interactions. In the light of this consideration the extreme variability of these molecules on the M cell surface has been interpreted as an effective way to generate a broad repertoire of M cell surface binding molecules to recognise bacteria borne lectins. M cell specific glycoconjugates have been successfully used as targets for oral and nasal delivery of antigens and particulate carrier. However, regional differences in the glycosylation pattern of M cells could influence the outcome of an antigen challenge. Studies in sheep have shown that the location in the gut in which antigen transport takes place may have profound effects on the immune responses. Both systemic and mucosal immune responses were observed when the vaccine vector was delivered to jejunal, but not ileal, Peyer’s patches. In the latter case only a systemic response was observed.

M CELLS AS THE MAIN PORTAL OF ENTRY FOR ENTEROPATHOGENS

In contrast with the positive role of M cells in the initiation of mucosal immune responses, their capacity for antigen sampling can facilitate invasion by potential harmful intestinal microorganisms. The risk is mitigated by the fact that these pathogens are directly delivered to areas of the immune system fully equipped to cope with such an emergency. Nevertheless, some microorganisms exploit M cells as an entry site to breach the mucosal barrier and establish local and systemic infections. The most dramatic evidence of such ability is provided by studies performed on Salmonella, Shigella, and Yersinia in different animal models. A detailed analysis of the various mechanisms used by these bacteria is beyond the scope of this article but has recently been reviewed elsewhere.

Origin and fate of intestinal M cells: facts and hypotheses

Intestinal epithelial cell maturation and differentiation is a well known phenomenon which is completed in a geographically well organised migration. The epithelium of each follicle derives from surrounding crypts, each crypt being a clonal unit, which are characterised by two distinct axes of migration and differentiation. Cells located on the villus side of the crypt differentiate into absorptive enterocytes, goblet, and enteroendocrine cells. Cells on the FAE side of the crypt move onto the dome, acquiring features of follicle associated enterocytes and M cells. The final differentiation of intestinal epithelial cells
takes place as they migrate in vertical bands to the apical
expression zone of the villus and FAE.10 12 Within this scenario
two different theories on M cell genesis in the FAE have
been formulated. Although it is accepted that enterocytes
and M cells have a common precursor it has been
postulated that M cells may originate in the crypts as a dis-
tinct cell lineage from stem cells via an independent differ-
etiation programme or from enterocytes on interaction
with the local lymphoid microenvironment.

Initially, the hypothesis that M cells are derived from
undifferentiated crypt cells was based on ultrastructural
studies and on the utilisation of 5-bromodeoxyuridine as a
proliferation marker. 5-bromodeoxyuridine labelled cells were
observed in crypts adjacent to the dome13 and within 24
hours they migrated at the dome periphery where they
acquired morphological and structural features of M cells.13 64
The most recent experimental evidence supporting
this idea mostly comes from studies on differential
expression of glycoconjugates on M cell membranes. It has
been demonstrated, in both the rabbit and mouse, that lec-
tin labelled M cells were not restricted to the dome of the
FAE but were also detected in crypts.65 66 These data indi-
Owing to the lack of well defined ultrastructural and histochemical
study has provided further evidence that
supports this view.66 Here it was also determined that the
random distribution of the sites where lymphocytes invade
the FAE did not correlate with the organisation of M cells,
and that a subpopulation of crypt cells is predetermined
as M cells before attaining their morphological and
functional features. More recently another morphological
and histochemical study has provided further evidence that
under certain experimental conditions, can
revert back to
the FAE did not correlate with the organisation of M cells,
and histochemical study has provided further evidence that
these immature pre-M cells would elude detection because
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delivered two different signals to enterocytes, one with the
ability to disassemble the brush border and the second to
transcytosis.67 This hypothesis was based on the
observation that the sole disorganisation of the brush bor-
der in Caco2 cells, via suppression of villin by specific anti-
sense RNA,68 69 was not sufficient to trigger transcytosis.
Nevertheless, cells with the apical and functional fea-
tures of M cells that were not in contact with lymphocytes
were observed in other experimental systems.22 74 In one
such case rapid appearance of operational M cells after
bacterial challenge in vivo was preceded by an increase in
the number of cells displaying intermediate phenotype
between enterocytes and M cells. Most of these cells, prob-
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Concluding remarks

M cells are important in regulating access of microorganisms and antigens to areas of the intestinal immune system and in shaping the immune response.

However, much remains to be learnt of the biology of this cell. The importance of finding the best way to induce protective mucosal immune responses is highlighted by the accepted view that the systemic immune response is not adequate to fight the vast majority of pathogens to which we are exposed throughout life. It is believed that over 95% of human pathogenic microorganisms target host cells after crossing epithelial barriers. For this reason it would be highly desirable to induce specific immunity at the site of invasion. The need to potentiate immunological defence at the mucosal level is further stressed by the fact that in adult individuals the area of the mucosal surfaces reaches the impressive size of 400 m². A better understanding of the mechanisms involved in the recognition and transepithelial transport of antigens, along with the molecular basis of M cell formation within the FAE, will represent an important step forward in the design of new strategies to improve oral delivery of biologically active compounds to the intestinal immune system.

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