In vitro studies are elucidating the receptor basis of human Peyer's patch M cell pathogen entry with a view to discovering new targets to enable oral vaccination with particle-entrapped antigens

Keynote review:

Intestinal Peyer's patch M cells and oral vaccine targeting

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Specialized M cells in the follicle-associated epithelium of intestinal Peyer's patches serve as portals for diverse particulates. Following antigen handover to dome lymphocytes, a protective mucosal antibody secretion ensues. One approach to oral vaccine delivery is to mimic the entry pathways of pathogens via M cells. The paucity of human tissue for *in vitro* investigation has hampered the discovery of M-cell pathogen receptors; however an *in vitro* human M like-cell culture model displays many expected phenotypic features. Comparative studies using microarrays reveal several novel M-cell surface receptors that could be used to potentially target orally delivered antigens.

Although intestinal Peyer's patches (PP) were first described by Johan Peyer more than 300 years ago, the detailed cellular structure of these groups of lymphoid follicles only began to be deciphered in the 1970s with the advent of microscopic techniques that permitted elucidation of component surface epithelial cell types [1]. PP populations vary with respect to species, anatomical location, with age/developmental stage and as a consequence of exogenous factors. In humans, the number of PP along the length of the gastrointestinal tract increases to ~300 at puberty and then declines thereafter [2]. PP are at their highest density in human ileum where they comprize 10–1000 individual follicles organized into discrete lymphoid structures overlaid by a follicle-associated epithelium (FAE). Individual PPs are an example of organized gut-associated lymphoid tissue (GALT). They are visible to the naked eye as rounded or elongated structures, apparent on the luminal surface of the intestine. Their average diameters range from an eighth of a centimetre in mice, to one centimetre in dogs and man, and up to tens of centimetres in ruminants. However, their borders are not distinct and can be difficult to identify macroscopically. PP and

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1359-6446/05/$ – see front matter PII: S1359-6446(05)03536-1

lymphoid follicles are also found in the human colon but their function remains unknown. Within the FAE, M cells (or 'microfold' or 'membranous' cells), enterocytes and goblet cells comprize subsets. In 1922, rabbit PP were shown to be sites of uptake of *Mycobacterium tuberculosis* [3] but this was dismissed as a non-specific process of little importance.

Up to 95% of pathogens cross epithelial barriers, therefore, attempts to manipulate specific immune responses at inductive sites, such as PP, could lead to new mucosal vaccines against established and emerging diseases [4,5]. Methods to generate specific immunity can be informed by understanding antigen-presenting mechanisms at these invasion site(s). Existing examples of mucosal inductive sites employed in this way include intradermal delivery of antigens to dendritic cells in human skin [6], as well as delivery of mucosal vaccines via nasal-associated lymphoid tissue [7]. It is anticipated, therefore, that a proper molecular and functional analysis of these portals of antigen exposure will lead to rational design of novel mucosal vaccine formulations comprizing appropriate targeting strategies and adjuvants.

Here, we review the role of the M cell in initiating mucosal immunity and update current knowledge of M-cell structure and function across a range of species as it impacts on the feasibility of targeting of oral vaccines. Recognizing that other sampling cells and mechanisms also contribute to mucosal immunity, we review the evidence for M-cell-specific uptake of a range of particulates including microbes. We discuss how these events relate to immunological outcome. Finally, we describe the potential of a human M-like cell model for production of new vaccine receptors for oral targeting.

M cells and immunity

In common with other M-cell-containing GALT, including tonsils and appendix, at one time PP were erroneously regarded by some as vestigial organs. However, this view has changed. The immunology of mammalian PP has been recently reviewed [8], specifically with respect to tolerance and food allergy [9] and also to parasite infection [10]. FAE-located M cells sample particulates from the luminal side of the gastrointestinal tract, presenting them to the lamina propria, which contains dense populations of lymphocytes, macrophages and dendritic cells. Using *in vitro* and *in vivo* methods, it has been shown that PP can absorb a wide variety of particulates, including proteins [11] and antigens [12]. This is important because the epithelium is considered a barrier to the entry of non-nutritional macromolecules from the lumen. General agreement on M-cell function fails to go much further than this. At the same time, the structure and function of PP express species-specific features, which contribute to host–pathogen biology. Although M-cell numbers are thought to be regulated by bacterial challenge, there are abundant PP-like follicles in the sterile small intestine of neonatal ruminants and pigs. These B-cell-rich follicles, which disappear by young adulthood, can achieve a mass equivalent to that of the thymus representing up to 1% of total body weight [13]. The principal contribution of such transient structures might be in the basic education of the immune system, including a contribution to the development of immunological tolerance [14]. A second mammalian population of PP-like lymphoid follicles tend to reduce with age, albeit much more slowly. Species-specific patterns of PP development have been described [13] but strict functional interpretation awaits elucidation.

BOX 1

Requirements for a successful targeted oral vaccine

- High entrapment efficiency in particulate formulation
- Antigen stability in particle retained
- Protection of antigen from intestinal metabolism
- Uptake of vaccine by M cells through targeting and/or adjuvants
- Antigen trafficking to dome lymphoid and dendritic cells
- Stimulate durable systemic and mucosal immunity
- Protect animal model against challenge
- Scale-up formulation process
- Phase I trial

M cells and other routes of antigen trafficking across the intestine

It is generally accepted that the epithelium overlaying PP and, more specifically, the M cell contributes in an active manner to antigen trafficking. Thus, in the case of neonatal large farm animals the rate of uptake by FAE could be of a magnitude that is enough to permit effective passive transfer of maternal immunoglobulins [15]. However, the proportional contribution to overall function of the immune system (as well as opportunities for vaccine strategy) is not well understood. As a portal of entry, M cells therefore represent generic sampling sites. Their principal role is to deliver exogenous (luminal) material to subepithelial compartment(s). Questions remain as to whether M-cell translocation of immunogens has a specific role in initial immune induction and also in the response to challenge of a primed system.

M-cell translocation of luminal material appears to represent a breach in the innate immune system. However, this might be of advantage to the host if such delivery is coupled to immune protection of a previously primed mucosal immune system. For example, M cells in PP are regarded as being instrumental in initiating mucosal immunity against pathogens invading across epithelial barriers [16]. From studies with IgA knockout mice, it is established that the production of secretory IgA in response to specific viral pathogens is an effective way to prevent subsequent attachment of the agent to the PP mucosa and that this protects against oral challenge [17]. Therefore, M-cell uptake of antigen and subsequent hand-over to professional antigen presenting cells appears to be a key