Targeting of whole killed bacteria to gastrointestinal M-cells induces humoral immunity in the female reproductive tract

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Recently, we demonstrated that oral delivery of whole killed bacteria, when agglutinated by an M-cell targeting lectin, resulted in an enhanced systemic and mucosal antibody response, as well as a protective immunity, against the gut pathogens Helicobacter pylori and Campylobacter jejuni. Importantly, this protection was achieved without the addition of exogenous adjuvant. Here, in this addendum, we extend this initial study by reporting on the vaginal antibody response induced by these vaccinations. These data show that the targeting of M-cells within the gastrointestinal tract also induces the secretion of antigen-specific antibodies (IgG and IgA) at a distal mucosal site, namely the vaginal mucosa. This observation raises the possibility that oral delivery of a whole, killed bacteria vaccine that target intestinal M-cells could potentially provide a strategy for inducing protective immunity against pathogenic bacteria that infect mucosal sites outside the gastrointestinal tract.

M-Cell Targeting of Whole Killed Bacteria Induces Protective Immunity Against Gastrointestinal Pathogens

M-cells are a specialized type of epithelial cell that are a vital and integral part of the immune system in the gastrointestinal tract. These M-cells, classically located at the luminal surface of the dome structure of Peyer’s patches in both man and mouse, have shortened and reduced numbers of microvilli enabling them to easily sample and transfer antigen in the gastrointestinal tract across the epithelial surface to the underlying immune cells.1

In an article published recently in Infection and Immunity, we presented the first evaluation of the effects of delivering whole killed (formalin-fixed) pathogenic bacteria to gastrointestinal M-cells on host protective immunity.2 To achieve this specific delivery, we utilized Ulex europaeus agglutinin I (UEA-I) which has the unusual property of uniquely binding the apical surface of M-cells in the mouse gastrointestinal tract. This lectin was used to agglutinate two important human pathogens, Helicobacter pylori which infects and colonizes the gastric mucosa and is the major cause of peptic ulcer disease and gastric cancer,3 and Campylobacter jejuni which infects the intestinal tract and is one of the major causes of diarrhoea.4 Oral delivery of these UEA-I agglutinated whole killed bacteria not only induced systemic (serum) and mucosal (intestinal) bacteria-specific IgG and IgA antibodies in these mice, but also protective immunity against both pathogens.2

By using a different, non-M-cell binding lectin to agglutinate H. pylori, we were able to show that M-cell targeting was essential for the observed effects and that particulate bacterial clumps, per se, were insufficient. Further, by identifying a strain of H. pylori that was not agglutinated by UEA-I, we were able to demonstrate that co-delivery of non-agglutinated bacteria with this lectin failed to elevate the recipient’s pathogen-specific humoral response. This indicates that the lectin was not simply acting as an immunostimulatory adjuvant, and therefore was logically

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exerting its effects by delivering associated bacteria to the recipients Peyer’s patches.

A notable feature of this approach was that protective immunity was induced by M-cell targeting, without the addition of an exogenous adjuvant. One of the greatest challenges facing mucosal vaccine development is the paucity of mucosal adjuvants that are safe and effective for use in humans. Obviously a strategy that avoids the requirement for such an adjuvant circumvents this problem.

Extending these Findings
to a Distal Mucosal Site

It has long been proposed that immune responses at mucosal surfaces are linked by a common, or integrated, mucosal immune system. Immunity generated at one mucosal site circulates via this system to provide an immune response, and potentially protection, at other mucosal sites. With this in mind, we evaluated whether gastrointestinal M-cell targeting of whole fixed bacteria could potentially have applications for inducing protective immunity against infections at mucosal sites other than the gastrointestinal tract. Using vaginal washings collected from the same experiments as in our previously published study, we measured the levels of bacteria-specific antibodies in the female reproductive tract. Mice that were immunized via the oro-gastric route with either UEA-I agglutinated H. pylori (Fig. 1) or UEA-I agglutinated C. jejuni (Fig. 2) showed significantly enhanced, bacteria-specific IgG and IgA antibody titers in their vaginal washings, compared to mice receiving non-agglutinated bacteria. This enhanced vaginal response was induced equally well by UEA-I agglutinated viable or formalin-fixed bacteria (Fig. 1). Hence, targeted delivery of whole bacteria to the gastrointestinal M-cells induced a humoral response at a distal mucosal site, namely the vaginal mucosa.

Potential Applications

A number of pathogenic bacteria cause disease in the female reproductive tract. Uropathogenic Escherichia coli (UPEC) is the primary cause of community-acquired urinary tract infections including cystitis and pyelonephritis; Mycoplasma genitalium causes pelvic inflammatory disease; Chlamydia trachomatis infection can result in salpingitis, ectopic pregnancy or infertility, while Neisseria gonorrhoeae is the causative agent of gonorrhea.

The type of immunity required for protection against these pathogens is varied. While protection against UPEC can be mediated by either T-cells or antibodies, genital C. trachomatis infections are believed to be cleared by a CD4+ T-cell dependent, antibody independent response. In our study, we demonstrated that M-cell targeting of fixed bacteria induced protection against both C. jejuni and H. pylori. As vaccine-induced protection against C. jejuni is mediated by antibodies, whereas protection against H. pylori is mediated by a CD4+ T-cell dependent, antibody-independent immune process, clearly M-cell delivery of fixed bacteria can induce effective cellular and humoral immunity. It is therefore possible that such an approach may have...
wide applicability for inducing protection against a range of infections of the female reproductive tract, although clearly this will require much further investigation.

**Conclusion**

In summary, and extending our previous study, we have shown that gastrointestinal targeting of M-cells with whole fixed bacteria can induce specific immunity at a distal mucosal site, importantly without the requirement for an adjuvant. This observation, plus our previous demonstration of protection against two different bacterial pathogens, suggests this approach could potentially be used for the induction of protective immunity against a range of infections of the female reproductive tract.