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## Leading article

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# Vaccines against gut pathogens

Many infectious agents enter the body using the oral route and are able to establish infections in or through the gut. For protection against most pathogens we rely on immunity to prevent or limit infection. The expression of protective immunity in the gut is normally dependent both on local (mucosal) and systemic mechanisms. In order to obtain full protection against some pathogens, particularly non-invasive micro-organisms such as *Vibrio cholerae*, mucosal immunity may be particularly important. There is a need to take these factors into account when designing vaccines targeting gut pathogens. Conventional parenteral vaccines (injected vaccines) can induce a degree of systemic immunity but are generally poor stimulators of mucosal responses. Thus, a basic prerequisite for designing novel vaccines against gut associated pathogens may be the requirement to induce mucosal and potentially systemic immunity.<sup>1</sup> The most effective way to induce local immunity against infectious agents has so far proved to be direct application of vaccine antigens to mucosal surfaces (oral or intranasal delivery).<sup>2</sup> The fact that we have so few effective oral vaccines shows that the induction of protective immunity through oral immunisation is not an easy goal to achieve as many antigens are poor oral immunogens. Here we will focus, using bacterial pathogens as examples, on some recent approaches being used to generate novel oral vaccines.

### Live vaccines

Oral vaccines can be based on either live or non-living antigens. The generation of modern live oral vaccines involves the construction of genetically defined attenuated micro-organisms capable of inducing immunity in a non-harmful way. The recent improved understanding of bacterial virulence associated gene function offers the possibility of introducing multiple, defined, attenuating and stable mutations into the genome of bacterial pathogens. Furthermore, the use of precisely attenuated bacterial vectors as carriers for recombinant heterologous antigens can lead to the generation of multivalent vaccines.<sup>3</sup>

#### LIVE ATTENUATED SALMONELLA AND SHIGELLA VACCINES

Perhaps the most advanced work of this type has been performed using salmonella species, in particular *Salmonella typhi*, the cause of typhoid. Killed whole cell typhoid vaccines and the purified *S typhi* Vi capsular polysaccharide confer some degree of protection after parenteral administration but fail to trigger strong cellular Th1 type T cell responses and local gut responses associated with protection.<sup>4</sup> A number of experimental live oral salmonella vaccines designed using modern technology are in

development.<sup>1-5</sup> Salmonella strains harbouring mutations in genes of the shikimate pathway (*aro* genes) have impaired ability to grow in mammalian tissues (they are starved in vivo for the aromatic ring).<sup>6</sup> Salmonella strains harbouring mutations in one or two *aro* genes (i.e., *aroA*, *aroC*) are effective vaccines in several animal models after single dose oral administration and induce strong Th1 type and mucosal responses.<sup>7</sup> An *aroC/aroD* mutant of *S typhi* was well tolerated clinically in human volunteers; mild transient bacteraemia in a minority of the subjects was the only drawback.<sup>8</sup> Th1 responses, cytotoxic T lymphocyte responses, and IgG, IgA secreting gut derived lymphocytes appeared in the majority of vaccinees.<sup>8,9</sup> In an attempt to render the vaccine even safer, an *aroA/aroC/htrA* triple mutant has been engineered (*htrA* encodes a stress protein associated with survival of salmonella in macrophages) which has been shown to be immunogenic in humans with no adverse effects or bacteraemia.<sup>10</sup> Other genetically modified *S typhi* strains are currently being evaluated in humans<sup>11,12</sup> and similar attenuation strategies have been used to construct live attenuated shigella and cholera vaccines.<sup>13,14</sup>

Live attenuated salmonella vaccines are useful vectors for the delivery of recombinant antigens to the immune system. Genes encoding protective antigens from different pathogens can be cloned and expressed in salmonella vaccine strains, which are then used to piggy back these antigens to the mucosal and systemic immune systems via oral immunisation. A variety of foreign antigens from unrelated enteric pathogens (e.g., shigella, *V cholerae*, pathogenic *Escherichia coli*, and helminths) have been expressed in salmonella vaccine strains, often with the induction of good immune responses, opening up possible routes to multivalent oral vaccines. A few limited clinical studies have already been attempted.<sup>15</sup>

### Non-living mucosal vaccines

The observed non-responsiveness of the healthy host to many non-living environmental and food antigens (including many experimental oral vaccines) may well represent a mechanism to prevent wasteful or deleterious immune responses. Despite the apparent selective nature of the mucosal immune system, some bacterially derived antigens, such as *E coli* labile toxin (LT) and cholera toxin (CT), can evoke potent systemic and secretory immune responses following administration to a mucosal surface (mucosal immunogens).<sup>16</sup> In humans, however, ingestion of microgram quantities of either LT or CT is sufficient to

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**Abbreviations used in this article:** LT, labile toxin; CT, cholera toxin.

cause the profound fluid secretions typical of traveller's and cholera diarrhoea respectively.<sup>17</sup> Nevertheless, there remains considerable interest in LT and CT, and their pentameric receptor binding domains, as vaccine antigens and as vaccine delivery vehicles. In humans, the non-toxic B subunit pentamer moiety of CT (CT-B) induces strong intestinal IgA antibody responses and long-lasting immunological memory after oral ingestion.<sup>18</sup> Based on this, recombinant CT-B has become an important component of recently developed oral vaccines against cholera and diarrhoea caused by enterotoxigenic *E coli*.<sup>19</sup> In parallel, novel ways of delivering toxin binding domains are now being realised. The demonstration that LT-B expressed in transgenic potatoes is immunogenic in mice and humans suggests that an edible vaccine against a diarrhoeal disease is not implausible.<sup>20</sup>

The receptor binding domains of LT and CT have also been used as antigen delivery systems. In mice, oral administration of antigens coupled to CT-B, either chemically or genetically, has in several systems been found to enhance both intestinal and systemic IgA immune responses against the CT-B coupled antigen.<sup>21</sup> Significantly, potent mucosal adjuvant activity has been associated with native CT and LT. In experimental animals, CT and LT have potent adjuvant properties which stimulate mucosal IgA and systemic immune responses to unrelated, non-coupled antigens after mucosal co-immunisation. Results in humans also suggest that wild type LT may have adjuvant properties.<sup>22-23</sup> In part, this adjuvant activity seems to be linked to the A subunit catalysed ADP-ribosylating action of CT and LT. To facilitate their use as mucosal adjuvants, several research groups have successfully attenuated the toxicity of CT and LT through genetic engineering of the enzymatic A subunit.<sup>24-25</sup> Although some mutations introduced into the A subunit of CT or LT were found to prevent toxin assembly or had no effect on enzymatic activity, certain mutations (e.g. LTK63) completely inhibited measurable enzymatic activity but did not perturb the binding properties, stability, or the  $\alpha$  ray structure of the toxin. Importantly, mutant toxins like LTK63 retained some of the mucosal adjuvant properties of the native toxin. After oral immunisation, LTK63 has been shown to promote mucosal and systemic antibody responses to a co-administered antigen such as tetanus toxoid.<sup>26</sup> The availability of non-toxic derivatives of LT and CT with some adjuvant activity should facilitate their evaluation in clinical trials and potentially enhance the efficacy of new oral vaccines.

### *Helicobacter pylori*: an important challenge

The association between *H pylori* and chronic gastritis, peptic ulcer disease and gastric carcinoma make it an important modern day gut pathogen. People infected with *H pylori* produce significant quantities of *H pylori* specific serum IgG, as well as IgA and IgG antibodies in the mucosa. Despite this strong immune response, *H pylori* remains in the gastric mucosa. Consequently, immunisation was initially dismissed and the main research focus was on prophylaxis. However, recent reports of promising vaccination studies in animals<sup>27</sup> and humans<sup>22</sup> have now appeared. It is widely accepted that, given the worldwide prevalence of helicobacter infection and the difficulties inherent in trying to eradicate it by antibiotic therapy, vaccination would be a preferable strategy. In general, research to date on the development of *H pylori* vaccines has focused on two major areas: the route of delivery and the selection of the antigen. Studies suggest that antigen delivered either orogastrically, rectally, or subcutaneously, in combination with an effective adjuvant can elicit a strong protective immune response in the host. Recent studies have also shown that antigen delivery by attenuated salmonella strains is also effective.<sup>28</sup> In a

controlled study, recombinant *H pylori* urease was administered in combination with active LT to *H pylori* infected volunteers. Although no change in gastric inflammation was observed, there was an increase in circulating urease specific IgA producing cells and a decrease in bacterial load. Unfortunately, the majority of volunteers who received LT developed diarrhoea.<sup>22</sup> Although not clinically practicable, this study demonstrated the feasibility of future effective vaccine development. New technologies including delivery methods, new antigens and non-toxic adjuvants should help us realise this goal. The search for protective *H pylori* antigens is still ongoing and has been limited by the lack of appropriate, well characterised antigens. The release of the *H pylori* genome sequence and analysis by many laboratories will potentially lead to the identification of novel antigens for *H pylori* vaccines. The predominantly Th1 immune profile in many infected patients may contribute to the immunopathology observed.<sup>29</sup> It may therefore be advantageous to modulate host immunity, such that vaccinated individuals mount a Th2 or Th0 response to infection. Further insights into appropriate vaccine design may come from the study of the immune response generated by those who spontaneously clear infection. This has been seen among infants and elderly people.<sup>30</sup>

### Conclusions

Work on the design of novel vaccines against gut pathogens is entering an exciting era. Our general improved understanding of the molecular basis of disease means that rational approaches can replace the more empirical approaches previously used. However, there are considerable technical barriers to be overcome. We need to understand how to deliver vaccine antigens orally in an immunogenic form and in a way that will avoid stimulation of inappropriate and potentially damaging immune responses. The next critical phase will be the continuing clinical evaluation of prospective vaccine candidates.

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