Surface warfare against pathogens using mucosal vaccines

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Human pathogens that initiate disease following infection of mucosal surfaces represent the single largest cause of infectious disease among the world's populations. The World Health Organization's (Geneva, Switzerland) *Report of Infectious Disease Deaths for 1995* indicates that more than 13 million deaths were related to infectious disease worldwide during that year. The majority of those deaths were caused by organisms that first make contact with, and then either colonize or cross, mucosal surfaces to infect the host. The most cost-effective means of preventing the morbidity and mortality associated with these organisms is vaccination. In this issue, Karen Robinson and colleagues[1] present an alternative approach to vaccine delivery in which a nonpathogenic, noninvasive, and noncolonizing Gram-positive bacterium, *Lactococcus lactis*, is used as a vaccine vector for fragment C of tetanus toxin. Mice orally immunized with this vaccine exhibit increased antigen-specific serum IgG and fecal IgA and protection against lethal toxin challenge.

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Traditional vaccine strategies that involve parenteral immunization do not prevent the initial interaction between the pathogen and the host at the mucosal surface. In fact, traditional vaccine strategies do not prevent infection, but instead resolve infection before disease ensues. In some cases, HIV for example, once the virus crosses the mucosal surface and enters the host cell—be that a dendritic cell, an epithelial cell, or a T cell—the host–parasite relationship is moved decidedly in favor of the parasite. In that case, as in many others, a vaccine strategy that does not prevent the initial infection of the host is unlikely to succeed.

Recently, a great deal of attention has focused on mucosal immunization as a means of inducing secretory IgA (sIgA) antibodies directed against specific pathogens of mucosal surfaces. The rationale for this is the recognition that sIgA constitutes >80% of all antibodies produced in mucosal-associated lymphoid tissues in humans and that sIgA may block attachment of bacteria and viruses, neutralize bacterial toxins, and even inactivate invading viruses inside of epithelial cells. In addition, the existence of a common mucosal immune system permits immunization on one mucosal surface to induce secretion of antigen-specific sIgA at distant mucosal sites. It is only now being appreciated that mucosal immunization may be an effective means of inducing not only sIgA, but also systemic antibody and cell-mediated immunity.

One limitation to mucosal immunization is that antigens administered via this route are frequently not immunogenic. This can be overcome, however, using attenuated mutants of bacteria as carriers of heterologous antigens. Initial studies in this area were conducted with avirulent mutants of *Salmonella* spp. as vectors, principally because of the natural pathogenesis of the organism and the behavior of appropriately attenuated mutants, which can interact with the lymphoid tissues in the Peyer's patches (associated with the gut) without causing systemic disease[2–4]. The B cells primed in these gut-associated lymphoid tissues then migrate to the mesenteric lymph nodes and undergo differentiation. These B cells enter the thoracic duct, then the general circulation, and subsequently seed all of the secretory tissues of the body, including the lamina propria of the gut and respiratory tract. IgA is then produced by mature plasma cells and is transported onto the mucosal surface, where it is available to interact with invading pathogens.

Oral immunization at the level of the gut mucosa can elicit production of secretory antibodies on all mucosal surfaces. This greatly enhances the potential of this technique for immunization against infectious diseases. The drawback to this approach is the potential of the carrier strain to cause fever and bacteremia, especially in individuals that may be malnourished or immunosuppressed. Other attenuated bacterial pathogens have also been examined as vaccine carriers, but less is known about their efficacy or safety[5,6].

An alternative approach that avoids the

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use of attenuated pathogens is the use of commensal bacteria as vaccine carriers[7]. Several theoretical objections have been raised to the use of commensal organisms in vaccines, most importantly the potential induction of immune tolerance following longterm exposure to target antigens continually released by organisms that colonize mucosal surfaces. Such induction of immune tolerance has not been observed experimentally.

In one example, Medaglini et al.[8] engineered the human oral commensal *Streptococcus gordonii* to express on its surface a 204-amino acid allergen from hornet venom as a fusion with the M6 protein of *Streptococcus pyogenes*. Both systemic and mucosal antibody responses against the allergen were detected in mice orally colonized with this recombinant Gram-positive commensal carrier.

In the present paper, Robinson and colleagues have taken this concept one step further using *L. lactis* as the vaccine carrier. Previous studies from their laboratory have demonstrated that mice inoculated parenterally or intranasally with *L. lactis* expressing fragment C of tetanus toxin (TTFC) develop systemic serum antibodies against tetanus toxin that protects against lethal challenge[9,10]. The current work extends this concept to oral immunization with *L. lactis* expressing TTFC. Immunization via the oral route resulted in increased TTFC-specific serum IgG and fecal IgA and protection against lethal toxin challenge. An intriguing additional finding is that formalin or mitomycin C killed *L. lactis* are as effective as live *L. lactis* in eliciting serum IgG anti-TTFC when administered intranasally.

One drawback to the use of *L. lactis* as a carrier for oral immunization is the number of doses required (7 intragastric inoculations with 5×10^9 cells each in the current study). TTFC is a highly immunogenic protein and less immunogenic proteins may require higher doses or more immunizations. There was also no indication that the T-helper (Th)1 arm of the immune response was activated when TTFC was expressed by *L. lactis*; this is in contrast to with the findings of Yamamoto et al.[11] in which TTFC expressed in an attenuated *S. typhimurium* elicited both Th1 (interferon-γ and interleukin-2) and Th2 (interleukin-10, but not interleukin-4 or interleukin-5) cytokine responses.

In vaccine development, as in life, there are no singular solutions. Vaccine strategies that elicit cell-mediated immunity may be preferred for some infectious diseases. In such cases, attenuated *Salmonella* spp. may be the best choice as a vaccine carrier. Other infectious diseases may benefit from primarily humoral responses, in which case a commensal streptococcus or Gram-positive bacterium such as *L. lactis* may be a better choice of carrier. The use of these bacteria as delivery vectors of heterologous antigens to the secretory immune system constitutes a promising approach for the development of new vaccines against a number of diseases. Even though there are unresolved questions about the use of this technique, the results obtained to date are encouraging, and there is great potential here for development of safe, effective, affordable vaccines.

1. Robinson, K., Chamberlain, L.M., Schofield, K.M., Wells, J.M., and LePage, R.W.F. 1997. *Nature Biotechnology* **15**:653–657.
2. Càrdenas, L. and Clements, J.D. 1992. *Clin. Microbiol. Rev.* **5**:328–342.
3. Curtiss, R. et al. 1994. *Dev. Biol. Stand.* **82**:23–33.
4. Levine, M.M. et al. 1996. *J. Biotechnol.* **44**:193–196.
5. Cirillo, J.D., Stover, C.K., Bloom, B.R., Jacobs, W.R., and Barletta, R.G. 1995. *Clin. Infect. Dis.* **20**:1001–1009.
6. Renauld-Mongenie, G. et al. 1996. *Proc. Natl. Acad. Sci. USA* **93**:7944–7949.
7. Fischetti, V.A., Medaglini, D., and Pozzi, G. 1996. *Curr. Opin. Biotechnol.* **7**:659–666.
8. Medaglini, D., Pozzi, G., King, T.P., and Fischetti, V.A. 1995. *Proc. Natl. Acad. Sci. USA* **2**:6868–6872.
9. Wells, J.M., Wilson, P.W., Norton, P.M., Gasson, M.J., and LePage, R.W.F. 1993. *Mol. Microbiol.* **8**:1155–1162.
10. Wells, J.M., Norton, P.M., and LePage, R.W.F 1995. *Int. Dairy J.* **5**:1071–1079.
11. Yamamoto M., et al. 1996. *Ann. N.Y. Acad. Sci.* **778**:64–71.

Entering the domain of neurotrophin binding

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The neurotrophins are a family of four structurally related peptide growth factors that regulate neuronal differentiation, survival, and synaptic transmission of both peripheral and central neurons. Their pivotal role in nerve survival has suggested they would be useful in the treatment of many types of neurodegenerative diseases; however, clinical therapeutic trials of these proteins have been disappointing, complicated by the relative difficulties of delivery and pharmacokinetics in the central nervous system. Now, the identification of neurotrophin receptor domains reported in this issue by Holden et al.[1] throws some light on the mechanism of receptor binding and provides a necessary first step for the eventual identification of therapeutically useful peptide mimetics of these proteins.

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4/5) comprise the neurotrophin family —a group of highly homologous, highly basic, peptide growth factors that circulate as homodimers. They regulate neuronal function by interacting with members of the family of Trk receptor tyrosine kinases, which have in their extracellular domains cysteine-rich and leucine-rich regions, and more membrane proximal immunoglobulin-like domains[2]. Each neurotrophin demonstrates selectivity of Trk activation, with NGF binding to Trk A, BDNF and NT-4/5 binding to Trk B, and NT-3 binding most avidly to Trk C. A second class of neurotrophin receptor, with distant homology to the tumor necrosis factor (TNF) family of receptors has also been identified. This receptor, p75, binds all neurotrophins, but lacks an inherent enzymatic activity.

Although some classes of neurons exhibit marked fidelity in Trk receptor expression, and thus neurotrophin responsiveness, other subsets of neurons can express multiple Trk receptors, or may exhibit different patterns of Trk receptor expression at distinctive developmental stages. Furthermore, the identity of cells that locally synthesize neurotrophins in the adult central nervous system (CNS) is still under intense investigation. Although some neurotrophin synthesis is target derived, local autocrine production by neurons, as well as paracrine production by astrocytes and microglia, has been hypothesized.

Several different approaches have identified immunoglobulin-like domains of the Trk A receptor tyrosine kinase as those critical for NGF binding. In the present study, Holden et al. demonstrate that coinjection of purified TrkA immunoglobulin-like domains with NGF prevents the biological response of local skin edema formation normally elicited upon NGF intradermal injection. These results confirm previous in vitro binding studies that mapped the immunoglobulin domains as the critical regions for Trk–neurotrophin interaction[3–5]. The utilization of an immunoglobulin-like domain for peptide growth factor binding appears to be a recurrent theme in ligand–receptor signaling by receptor tyrosine kinases, as the fibroblast growth factor receptors and the vascular endothelial

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