Slaying the Hydra all at once or head by head?

New candidate vaccines show promise in protecting against the diverse pathogens that cause diarrheal diseases.

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DIARRHEAL DISEASES ACCOUNT for three million deaths annually, mostly among children[1]. Overcrowded suburbs in expanding Third World cities, poor rural dwellings and refugee camps are sites where the lack of hygiene makes diarrhea an overwhelming public health problem. Furthermore, the industrialization of food processing that accompanies economic development promotes the emergence of enteric pathogens, such as enterohemorrhagic *Escherichia coli* (EHEC). Diarrheal diseases are a major global health problem but will they be defeated by cure or prevention?

The curative approach has had some successes, notably a 50 percent reduction in deaths caused by diarrheal infections through the implementation of oral rehydration therapy. But antibiotic treatment of diarrheal diseases has been hampered by the rapid spread of antibiotic-resistant pathogens. From the prevention perspective, limited resources combined with rapid population growth make speedy improvements in hygiene unlikely, leaving vaccination as the most effective and affordable strategy. There will never be a single antidiarrheal vaccine, but hopefully several vaccines protecting against different pathogens will be developed. The Hydra will not be slain all at once but head by head.

The major hurdle to developing vaccines for diarrheal diseases is diversity. Diarrhea is caused by parasites, viruses, and multiple species and serotypes of bacteria. Serotype/serogroup-specific protection is often the rule, which means a vaccine must raise an immune response against an impressive panel of antigens. Depending on the causative organism, immune protection may be mucosal or systemic, humoral or cellular. Often, the basic principles of this protection are unknown and correlates of protection are lacking. Potential vaccinees include diverse categories of individuals: tourists, military personnel and children in endemic areas. To meet the challenge of this diversity, academia and industry are generating an equally diverse battery of anti-diarrheal vaccines: oral, parenteral (injected), whole-cell killed, particulate, live attenuated and DNA-based. Such diversity of effort should be preserved to warrant future success.

The World Health Organization (WHO) has identified rotavirus, *Shigella*, *Vibrio cholerae* O1 and O139, enterotoxigenic *E. coli* (ETEC), and *Salmonella typhi* as the primary enteric pathogens to be controlled by vaccination. Next on the list are enteropathogenic *E. coli* (EPEC), EHEC and *Entamoeba histolytica* (the cause of amebic dysentery). To develop successful vaccines against these organisms, accurate figures for disease burden, acute and chronic complications, as well as mortality are crucial. Furthermore, there needs to be rapid evaluation of vaccine candidates in humans (especially live oral products), identification of endemic areas where phase III trials can be carried out, and early involvement of local health authorities. Encouragingly, vaccines against each of the five pathogens at the top of the WHO list are currently at different stages of evaluation.

Rotavirus is the most frequent cause of diarrhea, both in developing and developed countries. A quadrivalent oral vaccine based on a rhesus rotavirus is now available[2,3]. The vaccine is composed of four reassortants that express the outer capsid protein (VP7) of the four most common serotypes of human rotavirus. More than 20,000 children worldwide have already received three doses of the vaccine. Among vaccinees, 48–80 percent were protected against rotaviral diarrhea, a level comparable to that following natural infection.Even more promising, the vaccine elicited 70–95 percent protection against severe diarrhea. This is a considerable accomplishment, although this first generation vaccine (due on the market soon) is not likely to control transmission of rotavirus. But can we improve on nature with second generation rotavirus vaccines? It has recently been reported that mucosal IgA directed against an inner capsid protein (VP6) and antibodies against a nonstructural glycoprotein with enterotoxic activity are protective in a mouse model of rotavirus infection[4,5].

Vaccination with parenteral whole-cell killed *Shigella* has consistently failed, prompting the pessimistic view that there will never be a vaccine against *Shigella*. This view has recently changed[6]. A *S. sonnei* parenteral vaccine, composed of detoxified lipopolysaccharide conjugated to a toxoid has provided 70 percent protection during an outbreak of *S. sonnei* among Israeli army recruits[7]. In addition, live attenuated strains of *Shigella* that show limited capacity to invade mucosal tissues have been constructed[8] by eliminating genes of the aromatic amino acid biosynthesis pathway and/or virulence genes encoding cell to cell spread. One-dose oral *Shigella flexneri* 2a and *Shigella dysenteriae* type 1 vaccine candidates are under evaluation in phase I, II and soon phase III trials. The serotype-specificity of protection in shigellosis will necessitate construction of a trivalent vaccine composed of live attenuated mutants of *S. flexneri* type 2a, *S. dysenteriae* type 1 and *S. sonnei*.

The last few years have seen the spread of the cholera pandemic to South America and the appearance of a new serotype of *Vibrio cholerae* (O139) in Asia. Poor efficacy has led to the abandoning of the traditional whole-cell parenteral cholera vaccine in favor of oral vaccination strategies[9]. A three dose oral killed whole-cell vaccine that also contains recombinant cholera toxin B-subunit (r-CTB) has been evaluated in Bangladesh. After three years, the vaccine's protection rate was a surprising 60 percent, demonstrating that cholera infection can be significantly reduced by oral administration of a well tolerated product. Furthermore, the simplicity and low cost of the vaccine make it possible to produce in areas where cholera is endemic. In another approach, live attenuated mutants of *V.cholerae* have been constructed. The prototype (CVD103Hg^R) mutant of *V. cholerae* 01 Inaba (the classic biotype) does not express cholera toxin or other accessory toxins. It is safe, immunogenic and provides 100 percent protection

against Inaba and 60 percent protection against El Tor (currently the most prevalent biotype) in volunteers receiving one oral dose. A live attenuated mutant of El Tor (CVD111) has also been constructed[10]. Phase III trials of a bivalent (CVD103Hg^R/CVD111) live attenuated oral vaccine are underway. A safe and effective cholera vaccine protecting against *V. cholerae* O1 and O139 should be available soon.

An efficacious ETEC vaccine must elicit IgA antibodies in the gut mucosa against the surface appendages of ETEC strains and/or against their heat labile and heat stable (ST) toxins. Vaccine development has been hampered by the diversity of colonization factors and the poor immunogenicity of ST. An oral killed whole-cell ETEC vaccine composed of the five strains that express the most prevalent colonizing factors (together with r-CTB) is immunogenic and deserves to be tested further[9].

A parenteral vaccine consisting of the Vi polysaccharide capsular antigen of *S. typhi* is already commercially available. It is safe and protective in endemic areas, but is an example of the current limitations of pure polysaccharide vaccines: immunogenicity and immune memory are poor in young children. A conjugated vaccine composed of the Vi antigen and a toxoid is set to replace the pure Vi material. The 'old' live attenuated typhoid vaccine consisted of *S. typhi* Ty21a, a strain that was poorly characterized and inconsistently protective. It has been replaced by a new generation of *S. typhi* mutants with deletions in the *aro*C, *aro*D and *htr*A genes, which encode proteins that help bacteria survive inside tissues. Live attenuated oral vaccines of these mutants are immunogenic in humans and the results of phase III trials are eagerly awaited[11]. As two good typhoid vaccines (conjugated and live oral) are in the works, choosing one may be difficult. The major advantage of the Vi conjugate is that it is simple to integrate into the vaccination schedule.

With effective antidiarrheal vaccines on the horizon, new questions arise. Will there be sufficient funds to support clinical trials in the developing and developed world? How and at what stage of the clinical trials should manufacturers be involved? Will regulatory agencies tolerate the diversity of vaccines protecting against several pathogens, but still against a single syndrome? How can vaccination programs be organized so as to reach all children? How can a battery of new antidiarrheal vaccines be effectively introduced into the already busy schedule for child vaccination programs?

No doubt all of these hurdles will eventually be overcome. Let's not forget that slaying the Hydra was just one of the Twelve Labors of Hercules!

1. World Health Organization on-line world health report. URL http://www.who.org/whr/1996/exsume.htm
2. Midthun, K. & Kapikian, A.Z. Rotavirus vaccines: An overview. *Clin.Microbiol. Rev.* **9**, 423–434 (1996).
3. Glass, R.I., Lang, D.R., Ivanoff, B.N. & Compans, R.W. Rotavirus from basic research to a vaccine. *J. Infect. Dis.* **174**, S1–2 (1996).
4. Burns, J.W., Siadat-Pajouh, M., Krishnaney, A.A. & Greenberg, H. Protective effect of rotavirus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. *Science* **272**, 104–107 (1996).
5. Ball, J.M., Tian, P., Zien, C.Q.-Y., Morris, A.P. & Estes, M.K. Age-dependent diarrhea induced by a rotaviral non-structural glycoprotein. *Science* **272**, 101–104 (1996).
6. New strategies for accelerating Shigella vaccine development. *WHO Weekly Epidemiological Record* **72**, 73–80 (1997).
7. Cohen, D. *et al.* Safety and immunogenicity of investigational *Shigella* conjugate vaccines in Israeli volunteers. *Infect. Immun.* **64**, 4074–4077 (1996).
8. Phalipon, A. & Sansonetti, P.J. Live attenuated *Shigella flexneri* mutants as vaccine candidates against shigellosis and vectors for antigen delivery. *Biologicals* **23**, 125–134 (1995).
9. Holmgren, J. & Svennerholm, A.M. Oral vaccines against cholera and enterotoxigenic *Escherichia coli* diarrhea. In *Mucosal vaccines.* (Eds. Kiyono, H., Ogra, P.L. & McGhee, J.R.) pp 241–253 (Academic Press, San Diego, 1996).
10. Taylor, D.N. *et al.* Evaluation of a bivalent (CVD103Hg^R/CVD111) live oral cholera vaccine in adult volunteers from the United States and Peru. *Infect. Immun.* **65**, 3852–3856 (1997).
11. Tacket, C. *et al.* Safety of live oral *Salmonella typhi* vaccine strains with deletions in *htr*A and *aro*C, *aro*D, and immune responses in humans. *Infect. Immun.* **65**, 452–456 (1997).

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Alchemy for asthma

Techniques that induce allergen tolerance in asthma patients are not yet fully effective or free of side effects.

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ASTHMA IS A modern plague, affecting more than ten percent of children in many westernized societies. The prevalence of asthma and other allergic diseases has doubled in the last twenty years. Although much is known about the ubiquitous environmental antigens (known as allergens) that precipitate asthma and allergic disorders, the processes driving the increase in atopic diseases are not fully understood.

Allergens are usually soluble proteins with dimensions that allow penetration into the airways of the nose and lung (see figure). Allergen sources include house dust mites, domestic and farm animals, and grass or tree pollens. Major allergens from a particular source induce an allergic response in many individuals, whereas minor allergens produce disease less frequently. Most major allergens are extremely well characterized, with known protein sequences and even three-dimensional structures. Tiny amounts of allergen precipitate disease, and total yearly exposures to allergen may be measured in nanograms.

Atopic disease is initiated when allergen comes into contact with immunoglobulin E (IgE) bound to the high affinity IgE receptor (FcεRI) on mast cells and other cells of the immune system. Receptor crosslinking releases a mixture of inflammatory mediators and cytokines. Immediate inflammation and associated symptoms resolve and are followed by a second phase, which typically peaks six hours after antigen exposure.

Although the acute response to allergen is mediated by IgE, IgE production and the late phase reaction are modulated by T cells. Helper T cells may be classified into Th1 and Th2 types, according to the pattern of their cytokine production[1]. Although the classification is an oversimplification, in general, Th1 cells produce interferon-γ and enhance cellular immune responses, whereas Th2 cells produce interleukin (IL)-4 and favor humoral responses, IgE production and atopy. The propensity to react to antigen either with a predominant Th1 or a predominant Th2 response appears to be under genetic control. Th2 responses are also encouraged when antigens are presented at mucosal surfaces[2].

Immune tolerance is a distinctive feature of allergy. Many children spontaneously 'grow out' of their asthma. Skin test reactions to allergens decline steadily from the mid-teens, and the total serum IgE concentration at 50 years of age is half that at age 20 (ref.3). The mechanism of the decline is obscure. It is not seen in other immunoglobulin classes.

The combination of inappropriate immune responses, well-known antigens and immune tolerance is a promising mixture from which to forge treatment by vac-