INFECTIOUS DISEASE

Curbing cholera

Robert H. Hall

Colonization of the gut by virulent *Vibrio cholerae* is suppressed by probiotic-like activity of a live cholera vaccine candidate and *Lactococcus lactis* in two animal models (Hubbard *et al.* and Mao *et al.*, this issue).

Cholera is endemic in at least 47 countries, but it poses its greatest challenge in the form of explosive epidemics among vulnerable, at-risk populations in low-resource and inaccessible settings. Approaches to managing cholera epidemics have recently been revitalized and are increasingly being applied at scale. The influence of scientific evidence has been particularly important in building consensus on new action plans that seek a 90% reduction in cholera deaths by 2030 (*1*). Two reports in this issue by Hubbard *et al.* (*2*) and Mao *et al.* (*3*) propose new bacterial-based interventions to prevent virulent *Vibrio cholerae* colonization and infection in animal models.

In their study, Hubbard *et al.* (*2*) describe HaitiV, an engineered live orally administered cholera vaccine candidate constructed from strain HaitiWT, a member of the contemporary ascendant hypervirulent clade of *V. cholerae*. Nine sequence-confirmed genetic modifications were introduced to delete known factors associated with virulence, reactogenicity, and drug resistance, as well as to genetically stabilize the genome against reversion. When tested in an infant rabbit preclinical model of cholera, HaitiV robustly colonized the rabbit gut but did not cause the cholera-like diarrhea induced by its parent strain, HaitiWT.

The strong colonization of HaitiV inspired the investigators to test whether inoculation and intestinal growth of this live candidate vaccine provided resistance against subsequent challenge from the HaitiWT parental strain before the rabbit host developed an adaptive immune response. When animals were pretreated with the live attenuated HaitiV and challenged with the virulent parental *V. cholerae* 24 hours later, they did not develop the cholera-like diarrhea seen in animals that were not pretreated with HaitiV (Fig. 1, top). The authors repeated the experiment by challenging HaitiV-pretreated animals with virulent *V. cholerae* of a heterologous serotype, again showing protection by HaitiV at 24 hours. Heterologous protection across *V. cholerae* serotypes is important because serotype switching occurs in cholera. Killed HaitiV did not confer resistance against a virulent *V. cholerae* challenge in the infant rabbits, suggesting that the protective effects may be due to this live vaccine candidate also functioning as a competitive probiotic.

Hubbard *et al.* (*2*) make some key observations, although they do not elucidate the mechanism of HaitiV-mediated resistance to colonization by virulent *V. cholerae*. The authors used a genome-wide screen to identify transposon insertion mutants of HaitiWT capable of overcoming the protection of HaitiV-pretreated animals, but no consistently and specifically enriched mutants were identified in the virulent HaitiWT challenge strain that enabled it to break through the colonization resistance provided by HaitiV. As all experiments were short-term, the immunogenicity of HaitiV was not explored by Hubbard *et al.* (*2*) It will be fascinating to investigate the longevity of colonization resistance and its specificity across the range of cholera genotypes, surface antigens, and attachment factors, as well as to elucidate any role played by resident gut microbiota.

The probiotic-like colonization resistance conferred by *V. cholerae* HaitiV may fill an important gap identified by mathematical modeling in the armamentarium to fight cholera epidemics. The present landscape of licensed orally administered cholera vaccines includes a series of closely related killed vaccines and one live formulation. The first killed vaccine formulation was licensed in 1991 and has subsequently undergone extensive modification, technology transfer, prequalification, and stockpiling at the World Health Organization (WHO). This stockpile has become invaluable for cholera control. Over 25 million doses have been accessed and used in 19 countries since 2013, and killed oral cholera vaccines have shown safety, tolerability, acceptability, and cost-effectiveness (*1*, *4*). Although a powerful public health tool, killed vaccines confer poor protection to infants and young children, vulnerable groups that can spread cholera. The label requirement for two vaccine doses administered 7 to 14 days apart and the delayed onset of acquired protective immunity during a period of high infection risk can complicate the response in cholera emergencies (*1*, *4*). This problem would be resolved with the availability of a live single-dose vaccine that confers rapid protection through colonization resistance while acquired immunity develops (Fig. 1, top). The rapid-onset probiotic-like activity of HaitiV suggests a new functionality for live vaccines in cholera prevention. The only licensed live, single-dose cholera vaccine is CVD103HgR. This vaccine is currently being formulated for field deployment and WHO prequalification (*5*). Viability-dependent colonization resistance has not been reported for CVD103HgR. Thirty years of clinical experience and scientific knowledge separate CVD103HgR and HaitiV, and these strains differ in genomic background, biotype, serotype, and specific genetic modifications. The deep clinical, field, and regulatory experience behind CVD103HgR will be valuable if, after further studies on safety and efficacy, HaitiV embarks on the lengthy path of product development, clinical evaluation, licensure, formulation for the field, and, ultimately, deployment.

Although potentially devastating, cholera epidemics are not the only source of disease burden and often distract attention from the >90% of the 1.3 million to 4.0 million cholera cases that occur annually in regions where *V. cholerae* is endemic. Children living in cholera-endemic regions usually also live with poor sanitation, malnutrition, enteric enteropathy, persistent low-grade diarrhea, and frequent exposure to empirical antimicrobials. A healthy gut microbiota is associated with nutritional benefits and with resistance to enteric infection and enteropathy. Studies in Bangladesh indicate that the gut microbiota at the time

National Institute of Allergy and Infectious Diseases, U.S. National Institutes of Health, Bethesda, MD 20892, USA.
Email: robert.hall@nih.gov

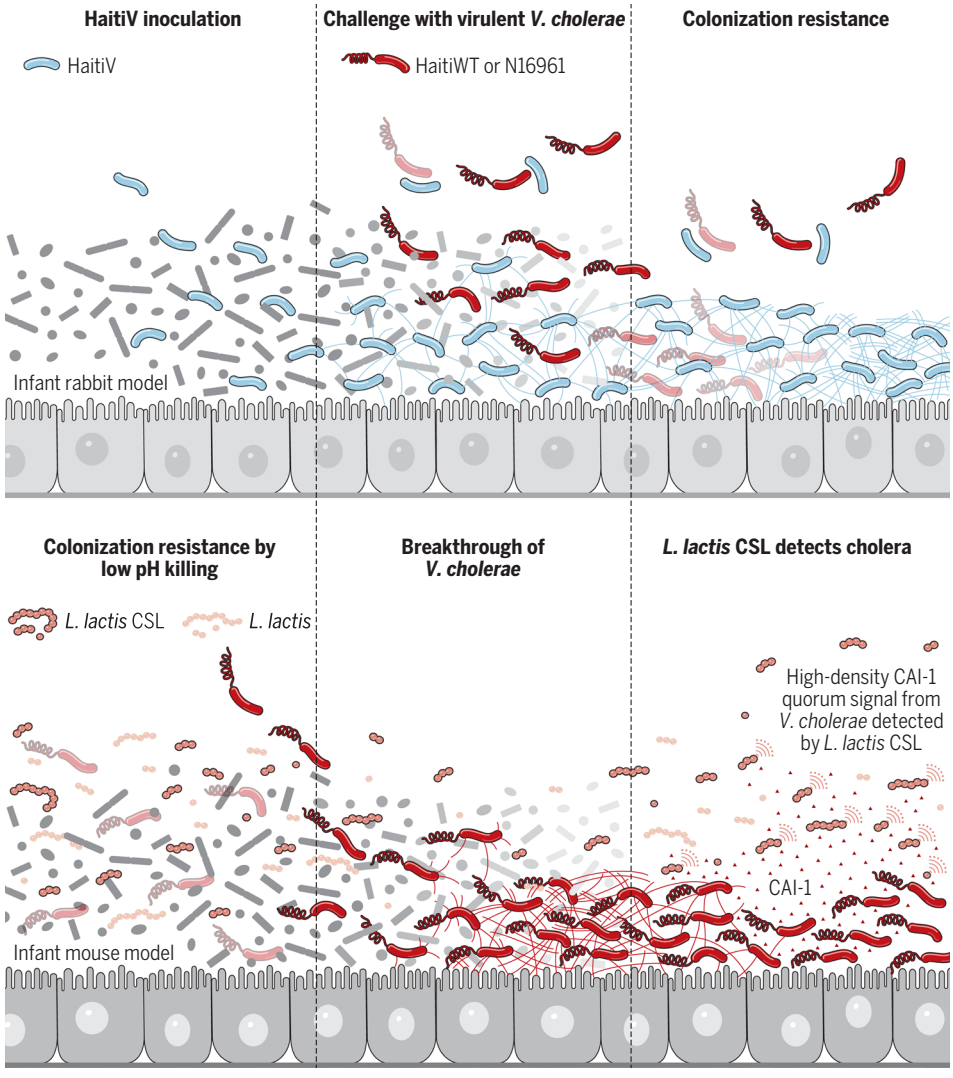

Fig. 1. Distinctive approaches to inducing cholera colonization resistance in animal models. (**Top**) The nonmotile vaccine strain of *V. cholerae* HaitiV (blue) demonstrates robust colonization of the infant rabbit intestine within 24 hours of inoculation (*2*). HaitiV-pretreated animals were protected from colonization and disease when subsequently challenged with either virulent homologous Ogawa serotype *V. cholerae* HaitiWT (red) or heterologous Inaba serotype *V. cholerae* N16961 (also red). Effects on commensal members of the rabbit gut microbiota (gray) were not determined in this study. The colonization resistance conferred by HaitiV occurred more rapidly than could be explained by the acquisition of acquired immunity. (**Bottom**) The presence of *L. lactis* (pink) and *L. lactis* CSL (pink with black outlines) inhibits the growth of *V. cholerae* and prevents colonization and disease in an infant mouse model (*3*). This effect was due to production of lactic acid by *L. lactis*, which alters the pH of the mouse gut. Potential negative effects of lactic acid on commensal members of the mouse gut microbiota (gray) were not determined in this study. In the event of breakthrough colonization by *V. cholerae* (red), the infection cycle proceeds through cholera toxin and toxin co-regulated pilus production, followed by increased bacterial cell density and expression of the quorum signal CAI-1. *V. cholerae* CAI-1 triggers expression of the β-lactamase reporter in *L. lactis* CSL, which can be observed following addition of nitrocefin to discharged feces, enabling visualization of the pathogenic *V. cholerae*.

CREDIT: A. KITTERMAN/*SCIENCE TRANSLATIONAL MEDICINE*

of exposure to *V. cholerae* is predictive of subsequent infection and that recovery from cholera involves a succession of ascendant species in the gut microbiota (*6*). A commercially available probiotic product has shown limited effectiveness at preventing acute diarrhea in children (*7*), but increasing knowledge about the enteric microbiota in health, disease, and recovery is opening opportunities for more rationally designed biotherapeutics that confer some protection against multiple pathogens.

Lactococcus lactis belongs to a family of lactic acid–producing bacteria used since ancient times to preserve foods through fermentation and more recently as a probiotic to promote general health and to deliver genetically engineered products orally. In new work, Mao *et al.* (*3*) investigate the potential of *L. lactis* to modify the intestinal milieu of the infant mouse gut into one antagonistic to the growth of *V. cholerae*. The authors demonstrated that metabolically active *L. lactis* inhibited proliferation of *V. cholerae* by two logs and increased the duration of survival of experimentally infected infant mice due to the low pH environment resulting from lactic acid production (Fig. 1, bottom).

The suppression of virulent *V. cholerae* could be helpful in maintaining intestinal homeostasis, given that *V. cholerae* infection appears to induce the expulsion of bacterial species of the phylum Firmicutes, of which *L. lactis* is a member (*8*). Furthermore, *V. cholerae* uses the type 6 secretion system to attack members of the host gut microbiota, to enhance pathogenesis, and to activate innate immunity in mice (*9*). It will be important to establish whether *L. lactis* affects the naturally occurring colonization resistance conferred by the gut microbiota in humans and whether feeding recuperating cholera patients with *L. lactis* may benefit the orderly and reproducible microbial succession identified by David *et al.* (*6*) during recovery in community- and hospital-based rehabilitation and nutrition programs. Mao *et al.* (*3*) chose a dose of 10^9 colony-forming units of *L. lactis* in infant mice, administered every 10 hours. If this turns out to be burdensome and costly when scaled to humans in cholera-endemic communities, then it could be appropriately prioritized with currently recommended measures for prevention and case management (*1*).

Diagnosing cholera, particularly asymptomatic cases, is important to guide and monitor control programs. Sensitive and specific dipsticks are widely used to detect cholera from rectal swabs in endemic areas and during fast-moving epidemics; the target product profile of improved cholera rapid diagnostic tests was recently updated (*10*). Mao *et al.* (*3*) describe bioengineering a diagnostic feature into *L. lactis* to produce *L. lactis* CSL, a live, enterically active bacterium that also acts as an in situ detector of *V. cholerae*. The detection system cloned into *L. lactis* CSL was a

hybrid of two-component histidine kinase systems. The sensor component used the transmembrane ligand-binding domain of CqsS, which binds the *Vibrio* genus-specific quorum-sensing molecule CAI-1. The signal transduction domain was provided by NisK, which is involved in regulating nisin production in *L. lactis*. The binding of CAI-1 was reported by derepressing extracellular expression of a β-lactamase drug resistance enzyme, which was detected using nitrocefin, a chromogenic cephalosporin substrate. When tested using in vitro coculture assays, *L. lactis* CSL reported a visible color change after a 30-min incubation with 10^8 live *V. cholerae* organisms. Fecal samples of *V. cholerae*–infected mice reported a positive signal after overnight incubation with nitrocefin, confirming β-lactamase secretion into the mouse gut (*3*).

The authors recognize the technical challenges and regulatory hurdles on the path to a product comprising *L. lactis* and *L. lactis* CSL for preventing, mitigating, and detecting cholera. A positive diagnostic signal will only be obtained in persons who ingested *L. lactis* CSL within the previous 10 hours and who are shedding large amounts of *V. cholerae*. The CAI-1 quorum signal may be repressed during the crucial early stages of cholera infection, with high expression occurring late in the *V. cholerae* infection cycle when expression of cholera toxin and toxin co-regulated pili are repressed (Fig. 1, bottom). The use of the drug resistance enzyme β-lactamase in a probiotic organism may raise regulatory issues. Intriguingly, in their study, Hubbard *et al.* (*2*) found that mutations in *V. cholerae cqsS* (encoding CqsS) were enriched in the gut of infected infant rabbits, suggesting that quorum sensing may play a distinct role in the pathogenesis of contemporary cholera strains.

Probiotics are widely accepted, command high prices, and hold remarkable product loyalty in many countries. Enhancing their effectiveness for specific health benefits is a promising area of active research and presents new challenges for regulation. Steps forward for clinical development could include collaborating with institutions with expertise in cholera, endemic disease, and enteric enteropathy in countries with a thriving probiotics industry.

The new ideas presented by Mao *et al.* (*3*) and Hubbard *et al.* (*2*) draw attention to the host intestinal compartment as a competitive environment that can be manipulated to increase resistance to infection without recourse to prophylactic antibiotics. In addition to developing a unique early-acting cholera vaccine candidate strain against virulent *V. cholerae* (*2*), establishing the mechanism of colonization resistance may also lead to novel biotherapeutics for other bacterial enteric infections. The finding that *L. lactis* can contribute to colonization resistance (*3*) may provide another avenue for probiotics to resist enteric infections during the potentially crucial period before the onset of a typical vaccine-induced immune response. The inclusion of a pathogen detection system in *L. lactis* CSL reveals the opportunities and challenges of monitoring intestinal health using a probiotic organism. These two important studies showcase innovative approaches to combating the scourge of cholera.

REFERENCES AND NOTES

1. Global Task Force on Cholera Control, *Ending Cholera—A Global Roadmap to 2030* (World Health Organization, 2017); www.who.int/cholera/publications/global-roadmap.pdf.
2. T. P. Hubbard, G. Billings, T. Dörr, B. Sit, A. R. Warr, C. J. Kuehl, M. Kim, F. Delgado, J. J. Mekalanos, J. A. Lewnard, M. K. Waldor, A live vaccine rapidly protects against cholera in an infant rabbit model. *Sci. Transl. Med.* **10**, eaap8423 (2018).
3. N. Mao, A. Cubillos-Ruiz, D. E. Cameron, J. J. Collins, Probiotic strains detect and suppress cholera in mice. *Sci. Transl. Med.* **10**, eaao2586 (2018).
4. S. N. Desai, L. Pezzoli, K. P. Alberti, S. Martin, A. Costa, W. Perea, D. Legros, Achievements and challenges for the use of killed oral cholera vaccines in the global stockpile era. *Hum. Vaccin. Immunother.* **13**, 579–587 (2017).
5. M. M. Levine, W. H. Chen, J. B. Kaper, M. Lock, L. Danzig, M. Gurwith, PaxVax CVD 103-HgR single-dose live oral cholera vaccine. *Expert Rev. Vaccines* **16**, 197–213 (2017).
6. L. A. David, A. Weil, E. T. Ryan, S. B. Calderwood, J. B. Harris, F. Chowdhury, Y. Begum, F. Qadri, R. C. LaRocque, P. J. Turnbaugh, Gut microbial succession follows acute secretory diarrhea in humans. *MBio.* **6**, e00381-15 (2015).
7. D. Sur, B. Manna, S. K. Niyogi, T. Ramamurthy, A. Palit, K. Nomoto, T. Takahashi, T. Shima, H. Tsuji, T. Kurakawa, Y. Takeda, G. B. Nair, S. K. Bhattacharya, Role of probiotic in preventing acute diarrhoea in children: A community-based, randomized, double-blind placebo-controlled field trial in an urban slum. *Epidemiol. Infect.* **139**, 919–926 (2011).
8. S. Monira, S. Nakamura, K. Gotoh, K. Izutsu, H. Watanabe, N. H. Alam, T. Nakaya, T. Horii, S. I. Ali, T. Iida, M. Alam, Metagenomic profile of gut microbiota in children during cholera and recovery. *Gut Pathog.* **5**, 1 (2013).
9. W. Zhao, F. Caro, W. Robins, J. J. Mekalanos, Antagonism toward the intestinal microbiota and its effect on *Vibrio cholerae* virulence. *Science* **359**, 210–213 (2018).
10. Global Task Force on Cholera Control, *Target Product Profile (TPP) for the Development of Improved Cholera Rapid Diagnostic Tests* (World Health Organization, 2017); www.who.int/cholera/task_force/cholera-rapid-diagnostic-test.pdf?ua=1.

Acknowledgments: R.H.H. is a member of the Oral Cholera Vaccine Working Group of the WHO Global Task Force on Cholera Control (OCV-WG-GTFCC).

10.1126/scitranslmed.aat9483

Citation: R. H. Hall, Curbing cholera. *Sci. Transl. Med.* **10**, eaat9483 (2018).

Science Translational Medicine

Curbing cholera

Robert H. Hall

Sci Transl Med **10**, eaat9483.
DOI: 10.1126/scitranslmed.aat9483