Emergence of different ctxB genotypes within virulent *Vibrio cholerae* populations accentuates the need to develop a vaccine that has the potential to protect against all cholera toxin genotypes. Oral administration of rCTB—alone and in combination with 2 dominant domestic killed whole cells of *V. cholerae* (O1 Ogawa El Tor and O1 Inaba El Tor) plus one standard *V. cholerae* (O1 Ogawa classic ATCC 14035)—has shown satisfactory protection as a potent vaccine candidate against toxigenic *V. cholerae*.

At the beginning of the 21st century, it is still important to treat cholera in epidemic or endemic areas of developing countries.1,2 Production of cholera toxin (CT) and its ability to adhere to the epithelial cells and colonize in the small intestine of the host are the most important virulence factors of epidemic *Vibrio cholerae* strains. 3,4 The CT is composed of two subunits: CTA is an enzymatic subunit that can activate adenylate cycles and increase the secretion of chloride and bicarbonate into intestinal lumen, and CTB is a pentameric nontoxic antigen with the ability to bind to GM1 ganglioside receptors on host epithelial cells, resulting in the entrance of CTA. Vaccination is a feasible and powerful strategy for prevention and control of cholera outbreaks. Different types of cholera vaccines have been developed including killed whole cell plus CTB.7-9 Several live-attenuated vaccine candidates have been developed including CVD103-HgR, CVD111, CVD101, CVD103, Peru-14, and Peru-15, which have been used in the clinical trials.5,6,8,10,11 These are single dose vaccines, which can elicit a high titer of serum vibriocidal antibodies because they can actively colonize the host intestinal lumen.6,12-14 The major defect of the engineered live vaccine strains is the probability of acquiring the enterotoxin gene through horizontal gene transfer. This, in turn, can transform the strains carrying toxins into virulent strains which could be troublesome, especially in epidemic areas.15,16 Vaccinations with oral killed whole cell plus CTB require multiple doses for a long-term immunity outcome. It can also be reasonably safe and has many advantages, including (1) prevention of bacterial colonization in intestinal lumen because of oral administration of vaccine, (2) improvement of adverse effects, and (3) low cost and ease of use.6,9,17 There have been many efforts to produce rCTB in prokaryotic and eukaryotic systems with no satisfactory results. Recently, we introduced a potent expression system consisting of BL21 (DE3) plus pAE_ctxB construct as an expression vector and have compared

**Keywords:** recombinant CTB, cholera toxin, native vaccine, rabbit, *Vibrio cholerae*

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A potent immunogenic cholera vaccine still remains a target.18 Two major strategies have been used to develop a cholera vaccine with long-term immunity: live-attenuated *V. cholerae* strains lacking the ability to produce CT7,8 and killed whole cell toxigenic *V. cholerae* strains in combination with CTB.7-9 Several live-attenuated vaccine candidates have been developed including CVD103-HgR, CVD111, CVD101, CVD103, Peru-14, and Peru-15, which have been used in the clinical trials.5,6,8,10,11 These are single dose vaccines, which can elicit a high titer of serum vibriocidal antibodies because they can actively colonize the host intestinal lumen.6,12-14 The major defect of the engineered live vaccine strains is the probability of acquiring the enterotoxin gene through horizontal gene transfer. This, in turn, can transform the strains carrying toxins into virulent strains which could be troublesome, especially in epidemic areas.15,16 Vaccinations with oral killed whole cell plus CTB require multiple doses for a long-term immunity outcome. It can also be reasonably safe and has many advantages, including (1) prevention of bacterial colonization in intestinal lumen because of oral administration of vaccine, (2) improvement of adverse effects, and (3) low cost and ease of use.6,9,17 There have been many efforts to produce rCTB in prokaryotic and eukaryotic systems with no satisfactory results. Recently, we introduced a potent expression system consisting of BL21 (DE3) plus pAE_ctxB construct as an expression vector and have compared
it with other expression systems. 14,18

The yield of recombinant CTB produced within this construct showed significant production of whole-cell Vibrio cholerae. 14,18 We have evaluated the biological and stability of our rCTB using GM1-ELISA assay. The results showed that rCTB has the ability to bind to its receptor. In addition, rCTB was shown to stimulate the immune response using a rabbit model. 16

Our previous results showed that two different ctb genotypes were present among V. cholerae isolated from recent outbreaks in Iran. 19,20 The circulation of inhabitant strains in a region can underline the significance of developing a potent local vaccine composed of our domestic strains with their own cholera toxin antigenic specifications for the control and prevention of cholera outbreaks in this region.

Our investigation of the level of anti-CTB IgG antibodies in a rabbit immunized with rCTB in combination with a different killed whole-cell toxigenic V. cholerae strain (O1 Ogawa El Tor and O1 Inaba El Tod) plus one standard V. cholerae strain (O1 Inaba El Tor) plus one standard V. cholerae strain (O1 Ogawa classic ATCC 14055) showed significant production of vibrio-like antigens. Furthermore, the immunized rabbits were challenged with the live toxigenic V. cholerae O115203 strain, and the fluid accumulation index was calculated by ileal loop assay. The results showed that rCTB alone and in combination with killed whole cell strains can provide protective immunity against V. cholerae and can be used as a potent vaccine candidate. 17

We will attempt to assess the immunological responses against a native whole cell plus CTB in combination with an additional mucosal adjuvant to improve its potential for stimulating mucosal immunity.

Disclosure of Potential Conflicts of Interest
No potential conflict of interest was disclosed.

References
1. Sak DA, Sak BB, Saha GB, Debroy S, Chakelto N, Laskar KS. The recombinant cholera toxin B subunit: a novel candidate for attenuation of oral carriers. J Mol Biol 2005; 350:21-30. PMID:15767379.
2. Hols P, Delcour J, Mercenier A, Kieny MP. Production of a filamentous phage encoding cholera toxin. Science 1998; 280:743-5. PMID:9648328.
3. Holm LR, Heuser JS, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1998; 280:743-5. PMID:9648328.
4. Arora AK, Saha GB, Debroy S, Chakelto N, Laskar KS. The recombinant cholera toxin B subunit: a novel candidate for attenuation of oral carriers. J Mol Biol 2005; 350:21-30. PMID:15767379.
5. Kieny MH, Wilder MK. CTB:la immunology: application in the development of cholera vaccines. Proc Natl Acad Sci U S A 1998; 95:7873-8. PMID:9648328.
6. Holm LR, Heuser JS, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1998; 280:743-5. PMID:9648328.
7. Holm LR, Heuser JS, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1998; 280:743-5. PMID:9648328.
8. Fonta M. Cholera vaccines. Clin Microbiol Rev 1998; 11:416-37. PMID:9714156.
9. Fonta M. Cholera vaccines. Clin Microbiol Rev 1998; 11:416-37. PMID:9714156.
10. Fonta M. Cholera vaccines. Clin Microbiol Rev 1998; 11:416-37. PMID:9714156.
22. Goto N, Masumoto J, Yamada Y, Isaka M, Matano K, Katayama T, Taniguchi T, Mizu Y, Okuma K, Tsuchida K. Safety evaluation of recombinant cholera toxin B subunit produced by Bacillus brevis as a nasal mucosal adjuvant. Vaccine 2000; 18:2644-71; PMID:10705520. http://dx.doi.org/10.1016/S0264-410X(99)00337-0.

23. Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan NH, Stanton B, Kay RH, Khan MU, Yunus M, Akhter M, et al. Field trial of oral cholera vaccines in Bangladesh. Lancet 1986; 2:124-7; PMID:3717995. http://dx.doi.org/10.1016/s0140-6736(86)91944-6.

24. Clemens JD, Sack DA, Harris JR, Chakraborty J, Neogy PK, Stanton B, Kay RH, Khan MU, Khan MR, et al. Cross-protection by B subunit-whole cell cholera vaccine against diarrhea associated with heat-labile enterotoxigenic Escherichia coli: results of a large-scale field trial. J Infect Dis 1988; 158:372-7; PMID:3442876. http://dx.doi.org/10.1086/316097.

25. Akhtar NH, Bakhshi B, Pourshafie MR, Sharifi A, Ghorbani M. Molecular diversity of CTX prophage in Vibrio cholerae. Lett Appl Microbiol 2011; 53:27-32; PMID:21982489. http://dx.doi.org/10.1111/j.1472-8229.2011.03270.x.

26. Dasttabapi-Roodbari A, Bakhshi B, Katadi M, Pourshafie MR. Comparative sequence analysis of mAb gene among Vibrio cholerae isolates from Iran with globally reported sequences. Lett Appl Microbiol 2011; 53:311-3; PMID:21757877. http://dx.doi.org/10.1111/j.1472-8229.2011.03088.x.