Evaluation of Immunogenicity to Bivalent, Whole-Cell, Oral Cholera Vaccines

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Cholera, mainly caused by *Vibrio cholerae*, is a life-threatening disease with high morbidity and mortality. Although a considerable progress has been made to develop effective vaccines, killed-whole cells by oral administration are still an attractive target for cost-effective vaccine with reasonable safety and immunogenicity. An effective cholera vaccine formulated with bivalent (O1 and O139) killed whole-cells had been developed and clinically used in Vietnam. Recently, this vaccine was reformulated to meet WHO requirements for international use and its safety and immunogenicity have been under investigation through clinical trials in Vietnam and India. For the clinical evaluation of cholera vaccine, we established and validated an optical density-based vibriocidal assay that has been a surrogate standard assay in the evaluation of cholera vaccine efficacy because of its good correlation with protective immunity. Then, the assay has been used in double-blind, randomized and placebo-controlled clinical studies for the efficacy test for the reformulated cholera vaccine with 153 subjects in Vietnam and 201 subjects in India, respectively. This assay has been transferred to the National Institute of Cholera and Enteric Diseases, Indian Council of Medical Research, Calcutta, India.

The optical density-based vibriocidal assay is indeed widely used in clinical trials, however it has limitations as vibriocidal titers are altered by incubation time. Furthermore the samples with same end-point titers could have potentially different vibriocidal kinetics. Recently, we have developed an improved agar-plate-based assay coupled with an automated colony counting system. This assay provides more stable and accurate vibriocidal titers than the conventional optical density-based assay and has approximately 24 times higher efficiency than a normal agar-plate-based assay. Notably, the newly-developed assay proved that some serum samples with the same endpoint titers have distinct vibriocidal kinetics that could not be distinguishable by the optical density-based assay. As a continuum of the assay development effort, we further improved this assay into the duplex semi-automated vibriocidal assay to simultaneously measure vibriocidal antibody titers against both O1 Inaba and O1 Ogawa strains in a single running. This assay can dramatically reduce the amount of human clinical sera required for the assay, time consumption, person-power and expenditure but at the same time increase sensitivity and accuracy of the assay.

The whole-cell cholera vaccine has been widely and clinically used, however exact mechanisms for the

protective immunity have been hampered due to lack of appropriate animal model. Thus, we established a mouse lung pneumonia model by intranasal administration of C57BL/6 mice with live *V. cholerae* and investigated the immunological property of an oral cholera vaccine, Dukoral™, which is the only licensed vaccine in the world at the moment. Our results showed that the cholera vaccine successfully protected mice from pneumonia-induced death. IgM antibodies specific to lipopolysaccharide (LPS) of *V. cholera* O1 Inaba were significantly enhanced in the sera of mice immunized with the cholera vaccine whereas anti-LPS IgG and IgA levels remained low in the same sera. Furthermore, anti-LPS IgM and IgA were detected in the mucosal lavage of lungs and secretory IgA against LPS was detected also in the nasal washes. And, the vaccinated mice produced significantly higher LPS-specific IgG- and IgA-secreting cells in both spleen and lymph nodes than those of the placebo control. In conclusion, the current animal model will provide further insight on understanding of the protective immune responses to advanced and new cholera vaccines.