[S5-2]

Not Lipopolysaccharide but Membrane Proteins Have Predominant Roles in the Innate Immune Responses to a Whole-Cell Vaccine against *Vibrio cholerae*

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Vibrio cholerae causes severe diarrhea leading to dehydration with high mortality and morbidity. Although a considerable progress has been made to develop effective vaccines, oral killed-whole cells are still an attractive target for cost-effective vaccines when safety and immunogenecity are in concern. In the present study, we investigated the immuological properties to heat-, ethanol-, or formaldehyde-killed *V. cholerae* in order to improve the efficacy of vaccines. Unlike other Gram-negative bacteria, the killed O1 Inaba, O1 Ogawa, and O139 potently activate Toll-like receptor 2 (TLR2) rather than TLR4 as determined using reporter cell lines, CHO/CD14/TLR2 and CHO/CD14/TLR4, that express human CD25 protein in response to TLR2 and TLR4 stimulation, respectively. Antibodies specifically blocking TLR2 or CD14 suppressed tumor necrosis factor-alpha (TNF-α) production by human peripheral blood mononuclear cells. Similar results were obtained in genetically-engineered mice showing that the killed cells failed to induce TNF-α production by bone marrow-derived macrophages from TLR2-deficient mice but not from TLR4-deficient mice. It appears that lipopolysaccharide (LPS) is not the only major immuno-stimulating component since the LPS purified from *V. cholerae* activates both TLR2 and TLR4 to the same extent. In contrast, membrane proteins isolated from *V. cholerae* showed more potent than its LPS in the activation of immune responses. Collectively, our results suggest that oral killed vaccine against *V. cholerae* predominantly activates TLR2 and the membrane proteins but not LPS play a major role in the innate immunity to *V. cholerae*.