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Poster Session V
Immunology, vaccination and host defences

IMMUNOGENICITY OF CONJUGATE BASED ON DETOXIFIED LPS ANTIGEN OF VIBRIO CHOLERAE O139

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Objectives

Lipopolysaccharide is a dominat protective antigen of *V. cholerae*. The aim of the study was to prepare glycoconjugate vaccine against *V. cholerae* O139. The immunogenicities of conjugate, induced IgM and IgG antibodies as well as levels of selected cytokines (IFN-gamma, TNF-alpha, IL-4, IL-6, IL-10 and IL-12) were determined by enzyme-linked immunosorbent assay.

Methods

Isolate of *V. cholerae* O139 (India, 1994), genotype *omp*U^{+}, *omp*W^{+}, *tox*R^{+}, *ctx*A^{+}, *tcp*A^{+}, *zot*$^{+}$, *hly*A^{+}, *ace*$^{+}$, *st*$^{-}$ was obtained from *The National Institute of Public Health, Prague, Czech Republic* and was used as a source of lipopolysaccharide. A glycoconjugate construct was based on attachment of hydrazine detoxified LPS to carboxylated BSA via its aminogroup. The immunogenicity of conjugate was estimated by enzyme-linked immunosorbent assay, testing of anti - LPS IgG and IgM antibodies and levels of selected cytokines.

Results

To prepare conjugate, we decided to use the detoxified LPS molecule, which retain their immunogenic properties. The number of conjugated dLPS per molecule of protein was 2-3. According our previous experiments, the way of attachment and size of the epitope is a crucial for effective activation of the immune system, enhancing the immunogenicity of the conjugate. *V. cholerae* O139 conjugate was applied to BALB/c mice in amount 2.5 μg of sacharide antigen per dose. The unconjugated dLPS elicited a moderate IgM response and very low IgG response comparable to pre-immune and saline control sera. Conjugate induced the significant increasing amounts ($P < 0.001$) of specific anti–LPS IgM antibodies only after 2nd dose. In contrast, the level of specific anti-LPS IgG antibodies, induced with conjugate progressively increased during the course of immunization. After the 2nd and 3rd dose, conjugate elicited a significant level of specific IgG ($P < 0.001$) compared to pre-immune and saline control sera, lasting at least 35 days ($P < 0.001$). Conjugate elicited high levels of IFN-gamma, IL – 4 and IL – 6 cytokines.

Conclusion

We confirmed that dLPS was functionally converted, due to the protein carrier effect, into a T-dependent antigen. T- cell dependent response was manifested by the effective switch of specific IgM-IgG antibodies. Our results demonstrate the efficiency of a conjugated dLPS in eliciting an IgG response and long - term memory function in mice.