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Poster Session V
Immunology, vaccination and host defences

IMMUNOMODULATIVE PROPERTIES OF VIBRIO CHOLERAE POLYSACCHARIDE-PROTEIN CONJUGATE VACCINES

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Objectives: The aim of the study was the preparation of *Vibrio cholerae* vaccine glycoconjugates and characterization of serological responses induced by different glycoconjugate constructs on the basis of detoxified lipopolysaccharides DeA-LPS. Also, were ascertained specific antibody isotypes and correlated them with vibriocidal effectiveness, as well as monitored the production of selected cytokines (IFN-gamma, TNF-alpha, IL-4, IL-6).

Methods: *V. cholerae* non-O1, serotype O135, strain NRC-66/171, chemotype a/II (according to Heilberg-Smith-Goodner scheme) and genotype *omp*W^{+}, *omp*U^{-}, *tox*R^{+}, *ctx*A/*ctx*A1^{-}, *tcp*$^{-}$ *st*$^{-}$, *zot*$^{-}$, *ace*$^{-}$, *hly*A/B^{+} was used for our study. The strain was isolated from Váh river in Slovakia. The serotype O135 is mostly found in east European rivers. This strain, NRC-66/171, was used as a source of surface LPS antigens. Following detoxification procedure, DaA-LPS were purified by size exclusion chromatography. Three glycoconjugates containing *Vibrio cholerae* DeA-LPS were prepared. One of them contained polysaccharide antigen DeA-LPS directly bounded to the bovine serum albumine protein using adipic acid dihydrazide spacer. The other types of conjugates contained DeA-LPS densely bounded to the synthetic polyoxazoline based carrier and to the protein. The immunogenicities (induced IgM, IgG, IgA antibodies) of all conjugates were determined by enzyme-linked immunosorbent assay in mice sera. Enhanced IgM vibriocidal activity of sera as well as production of IFN-gamma cytokine were evident here.

Results: Conjugates were subcutaneously applied to mice, but only saccharide-polymer-protein constructs induced high levels of specific anti- DeA-LPS antibodies. These two conjugates seem to be more effective and the serial increase of IgG levels during boosting of the antigen may indicate an isotype IgM-IgG switch. However, the serum vibriocidal activity was mediated by IgM only. Immunization of conjugates stimulated production of cytokines such as IFN-gamma, on the basis of which the predominance of Th1-immune response was concluded.

Conclusion: We confirmed that the multiple arrangement of short antigen in the vaccine construct is much more effective to cross B-cell receptors and evokes production of vibriocidal antibodies. The high density of the antigen attachment is important for effective activation of the immune system.