4 CONCLUSIONS

PRODUCTION OF INTEGRIN CD11b/CD18

PRODUCTION AND PURIFICATION OF CD11b SUBUNIT FOR PREPARATION OF ANTIBODY

- Four fragments of the subunit CD11b were produced and purified and then they were used for immunization of mice and for preparation of specific antibodies.

PRODUCTION OF THE INTEGRIN CD11b/CD18 IN INSECT EXPRESSION SYSTEM - S2 CELLS

- It has been demonstrated that even if low level of CD11b production was detected that S2 cells are not able to transport effectively this subunit to cell surface or to secrete its extracellular domain into medium and not even it has a signal peptide specific for S2 cells. Further it has been shown, that the level of production of CD18 subunit was not affected by usage of constitutive or inducible plasmid.

ANALYSIS OF THE INTERACTION OF RTX TOXINS WITH β_2 INTEGRINS – THE ROLE OF THE GLYCOSYLATION OF RECEPTORS IN BINDING OF RTX TOXIN

- Binding of CyaA to CD11b/CD18-expressing cells was lost upon deglycosylation of cell surface glycoproteins, suggesting that CyaA binding to the cell surface-expressed CD11b/CD18 integrin fully depends on its glycolsylation. It has been also demonstrated, that the deglycosylation did not affected formation of CD11b/CD18 heterodimer or its expression to cell surface.
- Toxin binding to CD11b/CD18-expressing cells was efficiently and specifically inhibited in the presence of only saccharide units that occur in the oligosaccharide chain of integrin molecule. This demonstrates, that CyaA directly recognizes the N-linked oligosaccharide chain of its β_2 integrin receptor.
- Cytotoxic action of CyaA depended on the glycosylation status of the CD11b/CD18-expressing cells.
- Glycosylation of β_2 integrin receptors plays a crucial role in binding and cytotoxic activity of others RTX toxins.

DELIVERY OF MYCOBACTERIAL EPITOPS BY RECOMBINANT CyaA TO ANTIGEN PRESENTING CELLS

- The delivery of the OVA:257-264 epitope to the MHC I molecules was strongly affected when the entire TB10.4 protein (TB10.4:1-96) was inserted into CyaA at position 336.
- The recombinant CyaA-TB10.4 toxoids were able to present the epitop TB10.4:20-28 on MHC I molecules with comparable or higher efficacy then the free minimal TB10.4:20-28 epitop.
- Except for CyaA-TB10.4:20-28-OVA, insertion at site 233 allowed a more efficient antigen presentation than insertion at position 336.
- Immunization with TB10.4 delivered by CyaA induces a strong TB10.4:20-28-specific CTL response.
- Toxoid CyaA-TB10.4:1-96-OVA induced strong proliferative response of splenocytes.
- Neither CyaA-TB10.4:15-33-OVA nor CyaA-TB10.4:1-96 was able to confer any significant protection against infection of *M. tuberculosis*.