CONCLUSIONS

The aim of my thesis was to prepare an "ideal" structure based on Tn antigen that would enable modulation of both adaptive and innate immunity. Tn antigen was selected because it is presented on the surface of tumour cells (as one of the tumour associated carbohydrate antigens) and because N-acetylhexosamines and their glycoconjugates were shown to modulate cytotoxic activity of natural killer cells.

To summarize:

Four types of immobilized MAGs were designed and successfully prepared:

- they showed high interaction potential with anti-Tn monoclonal antibodies
- they induced formation of high titre antisera specific to Tn antigen

Soluble MAGs of the same structure were not prepared and tested. The reason for it was partially disclosed by MD calculations. It showed that insertion of γ-Abu substantially (negatively) affected spatial behaviour of amino ends of individual branches of the growing molecule that hindered and prevented further "growth" on individual branches and thus preparation of target structures.

This situation resulted in the design and successful preparation of seven types of soluble comblike multiple antigenic glycopeptides. They differed by the number of Tn antigen on particular branch: 1, 2, or 3 groups for a branch, by an additional incorporation of a hapten group (DNP) or incorporation of a T-cell epitope. We proved that Tn antigen on this novel structural type of a carrier:

- was recognized by plant lectins giving the positive answer on the principal question about the steric accessibility of the presented Tn epitope in various arrangement on this dendrimeric structure
- was recognized by monoclonal anti-Tn antibody 83D4
- was recognized NKR-P1A and NKR-P1B receptors and showed unique and selective binding (compound 11)
- effectively modulated cytotoxic activity of NK cells
- induced formation of high titre antisera specific to Tn antigen

PERSPECTIVES AND APPLICABILITY

Original and promising results obtained on the modulation of adaptive immunity in mice model with compounds **13** and especially **15** as models of synthetic vaccine show that designed structures are perspective for further development as synthetic vaccine. In comparison with results published on synthetic vaccines with Tn antigen,[1,2] this concept is advantageous because it offers higher flexibility for optimalization and tailoring of the immunogenic properties including the incorporation of safe adjuvant. In this respect the

role of preimmunization on the production of IgM, IgG and IgA antibodies should be also clarified because obtained data indicate the immune system in the state of alertness give complex and stronger response. As a safe adjuvant we plan to use muramyldipeptide (MDP) analogues with minimized side-effects that are currently developed by RNDr. M. Ledvina's group at the IOCB. These structures are perspective because some of the analogues of MDP have been introduced into the clinical practice e.g. Romurtid™ and Likopid™, and hence the introduction of another analogue with better immunopharmacological profile would be less problematic.

Encouraging results obtained on the modulation of cytotoxic activity of NK cells via NKR-P1A and NKR-P1B receptors show that glycodendrimers might be selective ligands for individual receptors. Unfortunately, not enough data has been cumulated so far. Therefore, the extensive study focusing on the elucidation of the effect of spatial orientation and number and character of N-acetyl-D-hexosamine residues, and the effect of their clustering on the activation/inhibitory properties of these glycoclusters is necessary.

We also plan to use glycodendrimers for the preparation of mimetics of naturally occurring branched oligosaccharides. As, for example, in case of CD69 receptor we plan to continue in the work of A. Kovalová, Ph.D.[3] and use glycodendrimers bearing simple linear and branched oligosaccharides that she prepared to mimic natural pentaantenary oligosaccharide ligand derived from ovomucoid.[4]

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