SUMMARY

Biomolecular interactions form the basis of virtually all disease mechanisms in one way or another. Some of these interactions, especially protein-protein interactions are well characterised. Lipid-ligand protein-receptor interactions are far less understood, but this study shows how a resonant mirror biosensor can be employed to analyse such interactions using two examples. These lipid-protein interactions are also characterised by the manifestation of molecular mimicry between certain molecules.

In the first example, tuberculosis lipid antigens (mycolic acids) were immobilised on the biosensor surface using a cationic detergent. It is shown how these mycolic acids, or ligands, can be separated into their different subclasses using thin layer chromatography so that individual subclasses may be immobilised on the biosensor surface. The biosensor technique then allows discriminating between the different binding affinities of the subclasses with their relevant serum antibodies. In addition, it allows the characterisation of the relationship of the mycolic acids and their auto-antibodies with a very important structural mimic of keto-mycolic acid, namely cholesterol.

For the second example it is shown that the method optimised for the tuberculosis antigens is not generic and how this method had to be adapted for the use of Guillain-Barré syndrome glycolipid antigens: gangliosides. In this disease, the biomolecular antibody-auto-antigen interactions are characterised by molecular mimicry between gangliosides and lipopolysaccharides of *Campylobacter jejuni* infections. The measurement of binding of antibodies to auto-antigen G_{M1}-liposomes in a biosensor is shown to be a good model for studies of the idiotype network disturbances as gangliosides could be reproducibly coated on biosensor surfaces to report the specific binding of antibodies. A good positive control exists in the cholera toxin B subunit that binds with high affinity to ganglioside G_{M1}. Analysis of affinity constants reveals a good correlation with literature values. Lastly, it is shown that the immobilised G_{M1}-liposomes can be used to distinguish qualitatively and semi-quantitatively between Guillain-Barré syndrome patient and control sera. Besides providing the possibility of being a new diagnostic technique, it opens the possibility of studying the idiotype network and its role in auto-immunity much closer.

The idiotype network is a complex, tightly regulated network of antibodies, T- and B-cells. Disturbance of this equilibrium triggers auto-immune diseases like the Guillain-Barré syndrome. Molecular mimicry between self and foreign antigens is one mechanism by which auto-immunity may be triggered. In this study it is shown how a novel method of immobilising liposomes containing a glycolipid self-antigen on a resonant mirror biosensor may be used in unravelling the complexities of the idiotype network.

OPSOMMING

Biomolekulêre interaksies vorm die basis van byna alle siekte-meganismes op een of ander manier. Sommige van hierdie interaksies, veral proteïen-proteïn interaksies, is goed gekarakteriseer. Lipied-ligand proteïen reseptor interaksies word heelwat swakker verstaan, maar in hierdie studie word aangetoon hoe 'n resonans-spieël-biosensor aangewend kan word om sulke interaksies te analiseer deur gebruik te maak van twee voorbeelde. Hierdie lipied-proteïen interaksies word ook gekarakteriseer deur die manifestering van molekulêre nabootsing tussen sekere molekules.

In die eerste voorbeeld word tuberkulose lipied antigene (mikoolsure) gïmmobiliseer op die biosensor oppervlak deur van 'n kationiese detergens gebruik te maak. Dit word aangetoon hoe hierdie mikoolsure, of ligande, geskei kan word in hulle individuele subklasse deur gebruik te maak van dunlaag chromatografie sodat individuele subklasse geïmmobiliseer kan word op die biosensor oppervlak. Die biosensor tegniek laat dan die diskriminasie tussen verskillende bindings affinitieite van die subklasse met hul tersaaklike serum teenliggame toe. Verder laat dit die karakterisasie van die verhouding tussen mikoolsure en hul outo-teenliggame met 'n baie belangrike strukturele mimiek van keto-mikoolsuur, naamlik cholesterol, toe.

Vir die tweede voorbeeld word aangetoon dat die metode wat geoptimiseer was vir tuberkulose lipied antigene nie generies is nie en aangepas moes word vir die gebruik van Guillain-Barré sindroom glikolipied antigene: gangliosiede. In hierdie siekte word biomolekulêre teenliggam-outo-antigeen interaksies gekarakteriseer deur molekulêre nabootsing tussen gangliosiede en lipopolisakkariede van *Campylobacter jejuni* infeksies. Daar word aangetoon dat die meet van teenliggaam outo-antigeen binding aan gangliosied-liposome in 'n biosensor 'n goeie model is vir die bestudering van die idiotiepnetwerk-versteurings aangesien gangliosied betroubaar die biosensor oppervlak dek en teenliggaam binding rapporteer. 'n Goeie positiewe kontrole daarvoor bestaan in die cholera toksien B subeenheid wat bind aan gangliosied G_{M1} met hoë affiniteit. Analise van die affiniteits-konstantes toon goeie korrelasie met literatuur waardes. Laaste word getoon dat die geïmmobiliseerde G_{M1}-liposome gebruik kan word om kwalitatief en semi-kwantitatief te onderskei tussen Guillain-Barré sindroom pasiënt en kontrole sera. Behalwe die

verskaffing van 'n moontlike nuwe diagnostiese tegniek, skep dit die moontlikheid om die idiotiepnetwerk en sy rol in outo-immuniteit beter te bestudeer.

Die idiotiep netwerk is 'n komplekse, streng gereguleerde netwerk van teenliggame, T- en B-selle. Versteuring van hierdie ewewig sneller outoimmuun siektes soos die Guillain-Barré sindroom. Molekulêre nabootsing tussen self en vreemde antigene is een meganisme waardeur outoimmuniteit gesneller kan word. In hierdie studie word gewys hoe 'n nuwe metode van immobilisasie van liposome, wat 'n glikolipied self-antigeen bevat, op 'n resonans-spieël-biosensor gebruik kan word om die kompleksiteit van die idiotiep netwerk te ontrafel.