A fully synthetic carbohydrate vaccine based on gold nanoparticles

Fabrizio Chiodo,[1] Dodi Safari,[2] Marco Marradi,[1] Huberta A. Th. Dekker,[2] Yulong Shan,[3] Roberto Adamo,[4] Martina Lahmann,[3] Johannis P. Kamerling,[4] Harm Snippe,[2] Soledad Penadés[1]

[1]Lab. of GlycoNanotechnology, Biofunctional Nanomaterials, CIC biomaGUNE / CIBER-BBN, San Sebastián, Spain. [2]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands. [3]School of Chemistry, Bangor University, Bangor, UK. [4]Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, Utrecht, The Netherlands.

fchiodo@cicbiomagune.es

Since carbohydrates are usually poorly immunogenic, strategies have been developed to improve their immune response. Current advances in the identification and synthesis of carbohydrate epitopes have opened new ways to rationalize vaccine design. Several strategies for the production of synthetic carbohydrate-based vaccines have been developed that have overcome the hurdles encountered when using complex bacterial capsular polysaccharides.[1] The selection of a carrier in the preparation of a conjugate vaccine is another parameter in the design of carbohydrate vaccines.

This study explores the potential application of hybrid gold glyconanoparticles (GNPs) as vaccine candidates against *Streptococcus pneumoniae*, a major cause of invasive respiratory tract infections in both children and the elderly. The conjugation of biomolecules to metal nanoclusters has opened new opportunities in the design and synthesis of multifunctional and multimodal assembled systems for biomedical applications. Gold nanoparticles have been extensively explored because of their relative inertness, low toxicity, and easy manipulation, and because the chemistry of their surface is easy to control.[2] Gold surface can be simultaneously tailored with different ligands in a controlled way *via* thiol chemistry affording multivalent and multifunctional nanoparticles.[3],[4] In search for new conjugate vaccines not based on protein carriers, we here present gold nanoclusters as a versatile platform to construct a potential carbohydrate-based vaccine against *S. pneumoniae* type 14.

The branched tetrasaccharide Gal(β1-4)Glc(β1-6)[Gal(β1-4)]βGlcNAc, corresponding to the repeating unit of the pneumococcal type 14 capsular polysaccharide (Pn14PS), is able to induce anti-Pn14PS specific antibodies when conjugated to CRM_{197} and thus it was selected as candidate for the development of a synthetic conjugate vaccine against *S. pneumoniae* type 14.[5],[6]

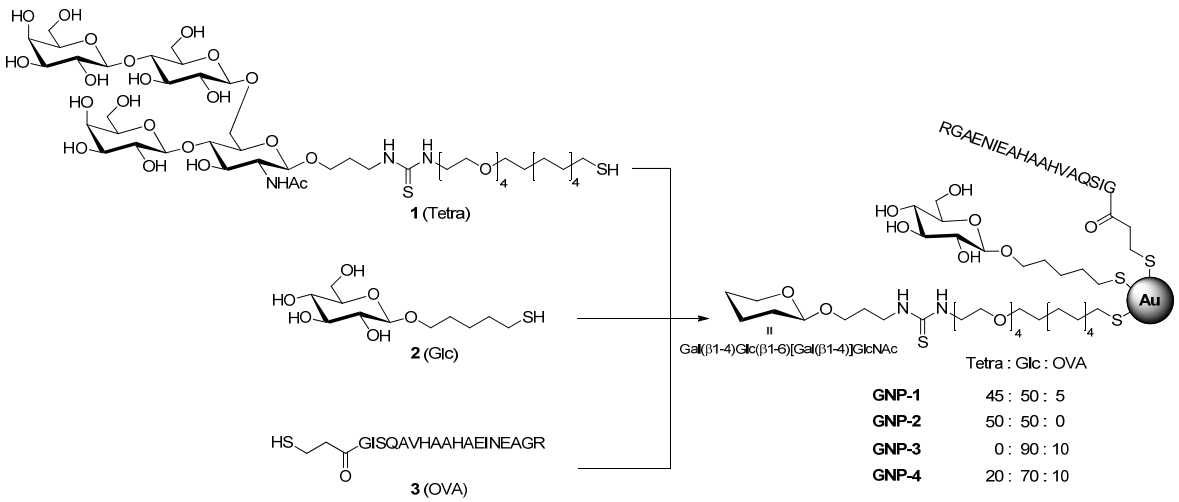
Antigenic tetrasaccharide Gal(β1-4)Glc(β1-6)[Gal(β1-4)]βGlcNAc, ovalbumin peptide-fragment $OVA_{323-339}$ which is well known as immunodominant T-cell epitope, and glucose were suitably functionalised with different thiol-ended linkers and employed as ligands for the construction of hybrid GNPs (scheme 1).

These biofunctional GNPs, having mean gold core diameters of ~2 nm, were used to immunise intracutaneously BALB/c mice. Depending on the density of the different ligands, GNPs coated with the tetrasaccharide conjugate induced significant levels of specific IgG antibodies that recognize both the native polysaccharide of Pn14 and the very same branched tetrasaccharide fragment of Pn14PS as determined by ELISA. Glucose was used as inner and inert component to assist water dispersibility and biocompatibility and to allow the tetrasaccharide moiety, armed with a long amphiphilic linker, protruding above the organic shell of GNPs.

Details on the synthesis of ligands, the one-step preparation of GNPs using different ratios of the ligands, and the techniques used for characterization (TEM, NMR, IR, UV-Vis) will be presented together with the experiments for evaluating the type-specific antibodies, opsonophagocitosis and cytokine levels after spleen cell stimulation. Although further optimization of vaccine efficacy is necessary, this study presents the first example of a fully synthetic carbohydrate vaccine based on nanoparticles that is able to induce specific IgG antibodies that react with native capsular polysaccharide.These results confirm that a suitable presentation of antigenic carbohydrates is essential to induce a specific immune response and should encourage the use of gold GNPs as new systems in the development of a synthetic carbohydrate-based pneumococcal vaccine.

References

[1] R. D. Astronomo, D. R. Burton, *Nat. Rev. Drug Discov.* **9**, (2010) 30
[2] M. Marradi, M. Martín-Lomas, S. Penadés, *Adv. Carbohydr. Chem. Biochem.* **64**, (2010) 211
[3] A. G. Barrientos, J. M. de la Fuente, T. C. Rojas, A. Fernandez, S. Penadés, *Chem. Eur. J.,* **9**, (2003) 1909
[4] R. Ojeda, J. L. de Paz, A. G. Barrientos, M. Martín-Lomas, S. Penadés, *Carbohydr. Res.*, **342**, (2007) 448
[5] F. Mawas, J. Niggermann, C. Jones, M. J. Corbel, J. P. Kamerling, J. F. G. Vliegenthart, *Infect. Immun.,* **70**, (2002) 5107
[6] D. Safari, H. A. T. Dekker, J. A. F. Joosten, D. Michalik, A. Carvalho de Souza, R. Adamo, M. Lahmann, A. Sundgren, S. Oscarson, J. P. Kamerling, H. Snippe, *Infect. Immun.*, **76**, **(**2008) 4615



*Scheme 1: One-step synthesis of hybrid gold nanoparticles (GNPs) incorporating different molar ratios of branched tetrasaccharide (Tetra; Gal-Glc-(Gal-)GlcNAc) (**1**), D-glucose (Glc) (**2**), and $OVA_{323-339}$-peptide (OVA) (**3**) conjugates. Reagents and conditions: $HAuCl_4$, $NaBH_4$, H_2O/MeOH, 2 h, 25 ºC. For clarity, all conjugates are depicted as thiols. The dimension of the gold nanoclusters is ca. 2 nm and is not in scale with the size of conjugates.*