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Extended Abstract

Rational Design, Synthesis, and Characterization of Glycoconjugates as Potential Vaccines against Tuberculosis †

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Tuberculosis (TB) is the first cause of death from infectious diseases worldwide. Only a single anti-TB vaccine is currently available for clinical use, but its efficacy is debatable. Conjugation of antigenic oligosaccharides, such as lipoarabinomannane (LAM), with antigenic proteins from *Mycobacterium tuberculosis* (MTB), has been recently proposed as a new strategy for developing efficient vaccines [1]. Glycosylation with α(1-6) polymannan has also been investigated in order to improve the biological activity of antigenic proteins [2]. This evidence was the rationale leading to the design, synthesis, and analytical characterization of the *neo*-glycoconjugates herein reported as potential vaccines against TB.

A number of semisynthetic glycoconjugates were prepared, starting from mannose, di-, and tri α(1-6) mannan analogues. Glycans were activated with a thiocyanomethyl group at the anomeric position to address protein glycosylation by a selective reaction with lysines of recombinant Ag85B (rAg85B). Since the immunogenicity of rAg85B was decreased upon glycosylation [3], the mutants K30 and K282 were designed by replacing lysines involved in the main T-epitope sequences, with an arginine residue (R) to prevent their glycosylation.

The effect of K30R, K282R, and K30R+K282R mutations on the T-cell activity of rAg85B was assessed by an immunological assay. The same test was carried out on the glycosylation products of the mutants. After glycosylation, the K30 mutants completely retained the original T-cell activity, thus resulting in antigenic carriers which might be suitable for the development of glycoconjugate-based vaccines against TB [4]. Moreover, the epitope of rAg85B involved in the interaction with antibodies from different sources was identified by proteolytic affinity-MS. The

affinity of rAg85B, mutants, and rAg85B-glycoconjugates for the monoclonal antibody anti-Ag85 was compared by SPR analyses to support the mutagenesis approach [5].

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