Production of semi-synthetic glycoproteins for the development of novel and effective glycoconjugate vaccines for tuberculosis (Immuno-HUB project)

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Tuberculosis (TB) is still the leading cause of death by an infectious disease worldwide: it kills more than 1 million people every year. TB prevention and treatment are hampered by the low efficacy of the sole vaccine available at present, the old bacille Calmette-Guérin, and by the increasing presence of antibiotic resistant *Mycobacterium tuberculosis* strains. Thus, new, effective vaccines against TB are urgently needed. The use of glycoconjugate vaccines is a successful strategy employed to fight various pathologies. In order to combine the antigenic properties of the proteins with the antigenic and/or immunogenic characteristics of the linked oligosaccharides (double hit approach), our work is aimed at the design and production of a series of subunit vaccines produced from selected recombinant antigenic proteins of *M. tuberculosis* (TB10.4 and Ag85B) and from their fusion (chimeric) proteins, chemically glycosylated and engineered to remove unwanted glycosylation sites. Starting from previous results (published in: Rinaldi F. et al. (2018) RSC Adv 8, 23171-23180), novel chimeric TB10.4-Ag85B proteins have been designed by inserting appropriate flexible linkers. Under optimized expression conditions, up to 10 mg of pure soluble chimeric proteins per L of *E. coli* culture medium have been now obtained. Glycosylation of the new chimeric proteins by employing different oligosaccharides (mainly arabinomannans) is now carried out using a novel approach. This project represents an innovative strategy for developing TB vaccines and shows the potential for application to other pathologies (e.g., COVID-19). This work is part of the Immuno-HUB Project (Immunoterapia: cura e prevenzione di malattie infettive e tumorali, project number T4-CN-02) supported by the Italian Ministry of Health.