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Biotechnological approach to designing a new antitubercular vaccine

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Background: The lack of an effective vaccine and the appearance of *Mycobacterium tuberculosis* MDR and XDR lineages are becoming a major public health problem, pushing the scientific community in creating new molecules that can be used as anti-tubercular vaccine. Starting from the Ag85B and TB10.4 native proteins, which are well known for their high antigenic activity, the Department of Drug Sciences, University of Pavia and the Department of Biotechnology and Life Sciences, University of Insubria Varese synthesized new glycoproteins Ag85B-MAN, Ag85B-MAN-MAN, TB10.4-MAN, TB10.4-MAN-MAN and mutant proteins Ag85B-K23R, Ag85B-K275R e Ag85B- K23R/K275R.
Subsequently, the molecules have been analyzed (at the S.C. Microbiology and Virology, IRCCS Policlinico San Matteo, Pavia) by ELISA and ELISPOT immunological assays using serum and lymphocytes from 24 patients with active tubercular infection (TB), 7 subjects vaccinated with Bacillus Calmette Guérin (BCG) and 8 healthy control subjects.

Material/methods: Immunological assays used in this study were indirect ELISA and cultured IFN-γ ELISPOT, to evaluate, respectively, the humoral response, by spectrophotometric quantifying of antibody responses towards the synthesized antigens, and the memory T-cell response, by quantifying the number of IFN-γ secreting cells after their expansion during a 10-day culture with the synthesized antigens before performing the standard ELISPOT assay. A statistic comparative study of collected data was conducted via median analysis, matched pairs Wilcoxon test and X^2 where $p<0.05$ means a statistically significant analysis.

Results: Serum obtained from both patients with active TB and vaccinated subjects reacted with the Ag85B-MAN-MAN glycoprotein, while no antibody reactivity was observed to the Ag85B-MAN glycoprotein. No humoral response was detected against the TB10.4 glycosylated protein. The glycosylation of TB10.4 seemed to slightly enhance the antigen-specific T-cell response in BCG-vaccinated subjects, with a decrement response in patients with active TB ($p>0.05$). Comparing antigen-specific T-cell response to the Ag85B protein and its glycosylated forms, BCG-vaccinated subjects and patients with active TB showed a decreased T-cell response, which was directly proportional to the increasing number of glycosylations. This might be attributed to the masking of antigenic sites in the native protein with sugars, following the glycosylation reaction ($p<0.05$). Preliminary data regarding the T-cell response against the Ag58B protein and its mutated forms showed an increase in immunogenic response, particularly to Ag85B-K23R/K275R, being the highest observed in BCG-vaccinated subjects.

Conclusions: *Ex-vivo* tests demonstrate that glycosylation may not have immunogenic effects, in the case of TB10.4, or variable effects, in the case of Ag85B. The increased T-cell response against Ag85B-MAN-MAN and Ag85B-K23R/K275R could be a good starting point to build new antigenic molecules, such as glycosylation mutant molecules using 2 mannoses. The collected data need validation with further biological tests.