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FIGHT-TB: DESIGN OF NOVEL VACCINE ANTIGENS TO FIGHT TUBERCULOSIS

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INTRODUCTION

Tuberculosis (TB) is the leading cause of death worldwide due to an infectious disease, causing around 1.6 million deaths each year (Fihure 1). This situation has become more complicated by the emergence of drug-resistant M. tuberculosis (Mtb) and HIV-TB co-infection, which has significantly worsened TB prognosis and treatment. Vaccination has been considered the best approach to reduce the TB burden. Since 1923, the Bacillus Calmette–Guérin (BCG) vaccine, an attenuated form of Mycobacterium bovis, remains the only licensed vaccine; Its efficacy against severe and extrapulmonary forms of paediatric TB is well recognized, but highly variable and poor protection at all ages against pulmonary TB remains a major concern [1]. Therefore, a safer and more effective vaccine to replace BCG or BCG-prime boosting is urgently required.

Following the establishment of primary infection, infected dendritic cells (DCs) and recruited monocytes transport Mtb to draining lymph nodes, which initiate T-cell priming and acquired immunity. T lymphocytes, in particular CD4+ T helper 1 (TH1) lymphocytes, and IFN-γ produced by these cells are necessary to control Mtb. Mtb infection elicits both effector and memory T cells. TB vaccine research has focused largely on identifying strong IFN-γ/TH1-inducing vaccine candidates for several years. Likewise, polyfunctional T cells that co-express multiple TH1 cytokines (TNF-α, IL-6, and IL-12) have been considered qualitatively superior to T cells expressing a single cytokine and are being investigated with much interest in ongoing vaccine trials. Although strong T-cell-stimulating antigens induce robust protective immunity in mice, these antigens cannot induce complete sterilizing immunity . It has been suggested that Mtb subverts CD4 T-cell dependent immunity by delaying initiation of T-cell responses via modulation of DC functions and survives in a dormant form [2,3].

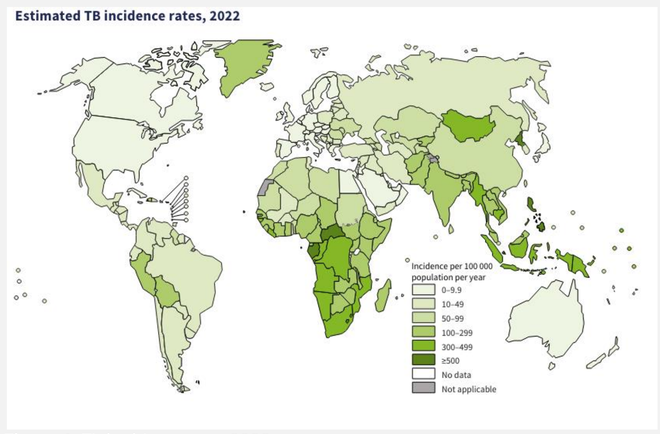


Figure 1. Tb incidence rate 2022.

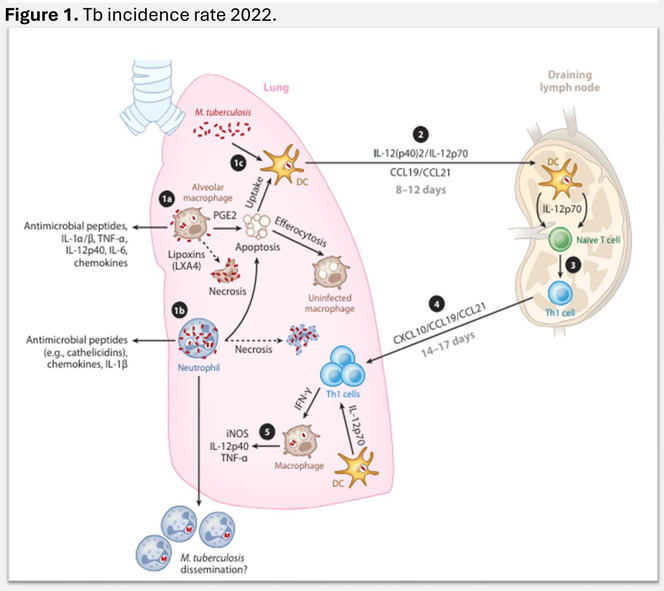


Figure 2. Mycobacterium tuberculosis infection (figure adopdet by https://doi.org/10.1038/nrdp.2016.76)

Therefore, early activation and migration of DCs to draining lymph nodes together with stimulation of T cells are key factors for inducing effective and durable protection against Mtb infection. These observations suggest that a mycobacterial antigen that elicits effective host protective immunity via DC activation is a promising target for development of a TB vaccine [3].

METHODS

The main purpose of FIGHT_TB project is to use structural vaccinology (SV) as a rational tool to improve immunogenic properties of some Mtb antigenic proteins (table 1) either to replace or boost BCG vaccine.
Structural vaccinology or structure-based antigen design is a rational approach that uses three-dimensional structural information to design novel and enhanced vaccine antigens.

The typical SV approach (Figure 3), involves the determination of the three-dimensional structure of the antigen or antigen–antibody complex using structural biology tools. This is achieved by combining the benefits of RV, with advancements in X-ray crystallography, NMR spectroscopy and single particle Cryo-Electron Microscopy, and novel computational approaches [4, 5].

Structural vaccinology offers a tangible tool to antigen improvement and the development of multi epitope vaccines. So FIGHT_TB combines different methodologies, like molecular and structural biology and chemical synthesis, with computational and experimental tools aimed at epitope identification.

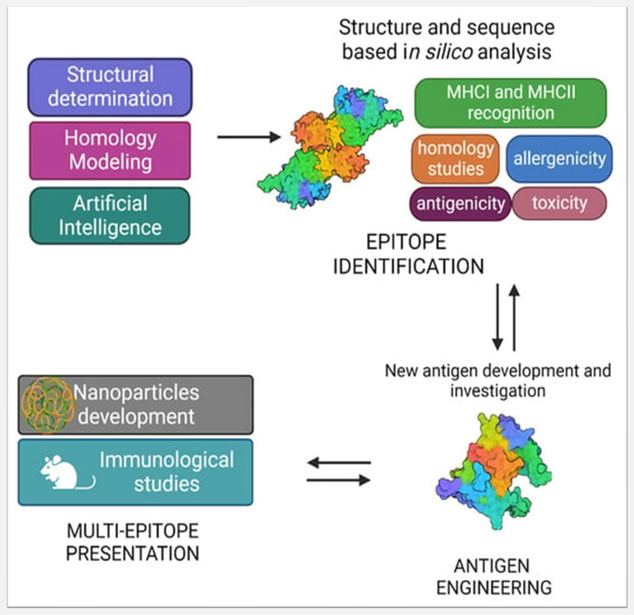


Figure 3.Structural vaccinolofy approach

OBJECTS of the research

We will focus on four proteins [6,7] that shown a promising vaccine potential and constitute valid starting points for antigen improvement (Table 1) .

Mtb protein	Putative function/annotation	Immunogenic function	Structural information
Rv2299c	Mycobacterial Chaperone **HtpG**	DC maturation Boosting BCG when fused with ESAT6	No structure available
Rv2450c	Cell Wall hydrolase/Virulence Factor **RpfE**	DC maturation	4CGE The recombinant Rv3463 is folded and Stable.
Rv1009	Cell Wall hydrolase/Virulence Factor **RpfB**	DC maturation	4KL7, 4KPM, 4EMN, 3EO5, 5E27
Rv3463	Putative oxidoreductase	Macrophage-activator Bactericidal activity	No structure available

Table 1. Protein antigens involved in this project

Production of the rationally designed antigen: Peptide synthesis

We used computational approaches to identify the most immunogenic region of Rv3463, we identified the fragment **Rv3463 68-96** able to intercat with HLA.
Mimicking natural antigens through peptides which mimic their epitope region is a promising strategy, named as (mimotope)-based, for vaccine development. In order to identify the epitope of the fragment , we synthesized a library of overlapping peptides (figure 5), with specific length and specific offset covering the entire fragment.

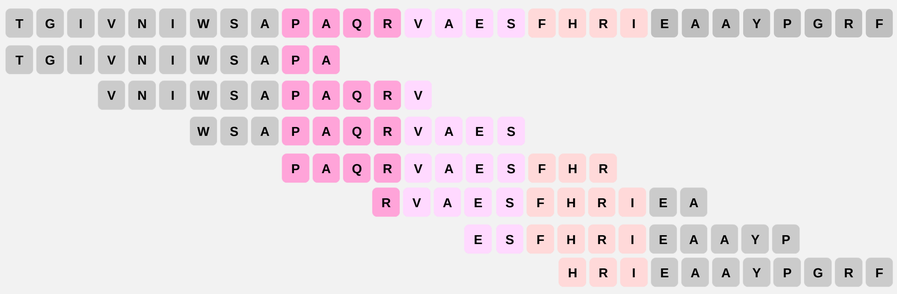
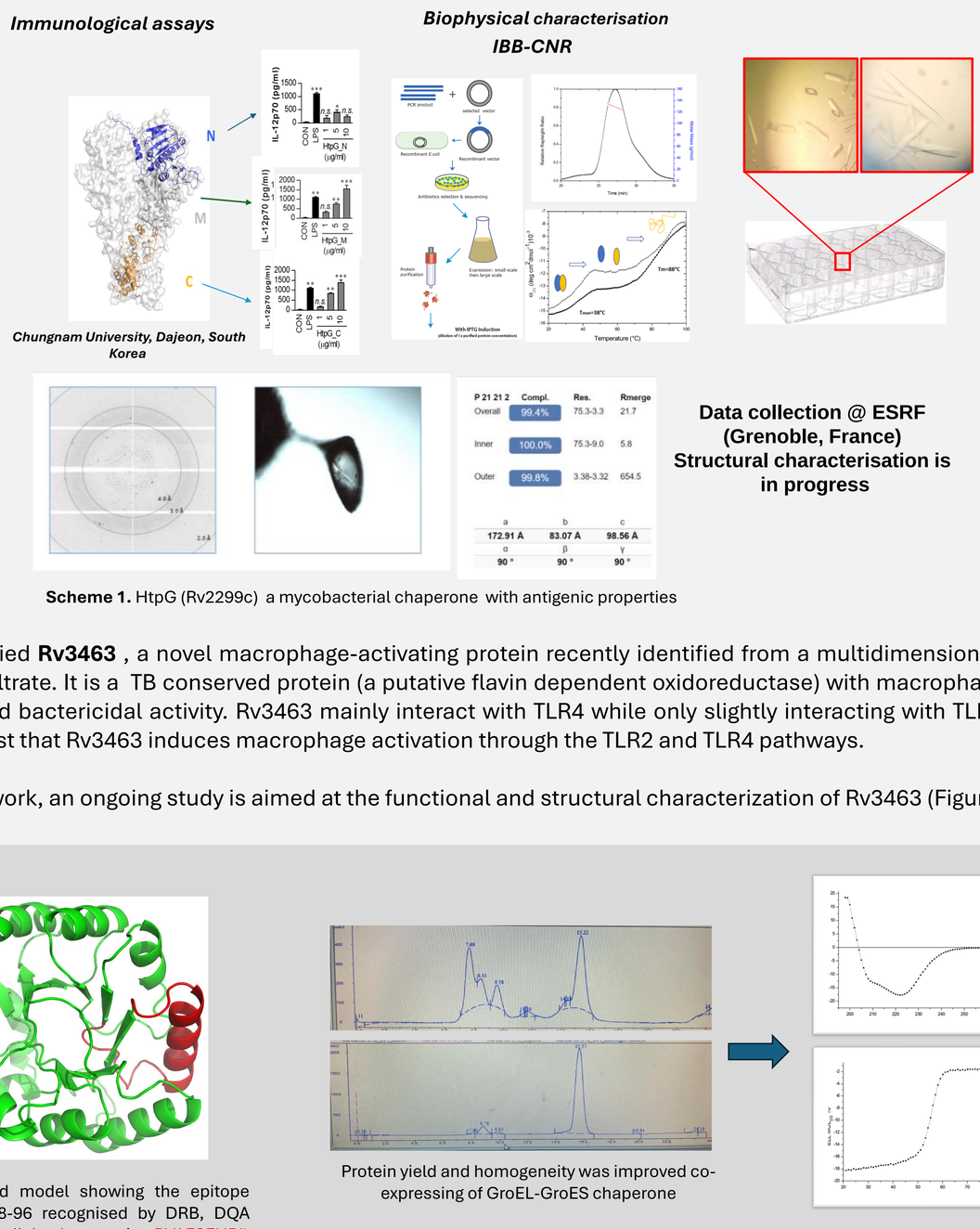


Figure 5 . Overlapping peptide library

Peptides synthesis was performed according to the solid phase approach using standard Fmoc methodology by CEM liberty blue 2.0 automated microwave synthesizer. The peptides purification was achieved using a preparative RP-HPLC C-18 bonded silica column. The purified peptides were 98% pure as determined by analytical RP-HPLC and molecular weights were confirmed by ESI-MS.

RESULTS

To date we focused our attention on **Rv2299c**, a mycobacterial chaperone named also HtpG. its activity depends on its nucleotide-binding state, we planned to solve the structure of HtpG, still unavailable, in its different nucleotide-binding states. To reach this goal, we performed a biophysical characterization and crystallisation trials by using a high-throughput crystallisation facility available at CNR [6]. Structural characterisation is still in progress (Scheme 1).



Scheme 1. HtpG (Rv2299c) a mycobacterial chaperone with antigenic properties

Then we studied **Rv3463** , a novel macrophage-activating protein recently identified from a multidimensional fraction of Mtb culture filtrate. It is a TB conserved protein (a putative flavin dependent oxidoreductase) with macrophage activating properties and bactericidal activity. Rv3463 mainly interact with TLR4 while only slightly interacting with TLR2 [7]. These results suggest that Rv3463 induces macrophage activation through the TLR2 and TLR4 pathways.

In this framework, an ongoing study is aimed at the functional and structural characterization of Rv3463 (Figure 4).

AlphaFold model showing the epitope region 68-96 recognised by DRB, DQA and DQB alleles (core region RVAESFHRI)

Protein yield and homogeneity was improved co-expressing of GroEL-GroES chaperone

Figure 4 . IBB-CNR/UNISA → Recombinant protein expression and its biophysical characterisation

CONCLUSION

Aims to improve the immunogenic properties of the investigated proteins using a structuralvaccinology approach. Structural vaccinology combines information from immunology with the benefits of the structural biology.
Indeed, structural data are used to determine the epitope region of each antigenic protein, either through structure-based prediction or through experimental epitope identification. This information is key to the re-design of vaccine antigens for a selectively enhancement of antigenic properties, using several strategies like (i) conjugation of antigens stimulating different immunomodulatory roots and (ii) multi-epitope presentation, a strategy that typically enhances the immune response, (iii) design of cross-reactive antigens, able to act on more bacterial species.

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