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Review

Immune recognition surface construction of *Mycobacterium tuberculosis* epitope-specific antibody responses in tuberculosis patients identified by peptide microarrays

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SUMMARY

Background: Understanding of humoral immune responses in tuberculosis (TB) is gaining interest, since B-cells and antibodies may be important in diagnosis as well as protective immune responses. High-content peptide microarrays (HCPM) are highly precise and reliable for gauging specific antibody responses to pathogens, as well as autoantigens.
Methods: An HCPM comprising epitopes spanning 154 proteins of *Mycobacterium tuberculosis* was used to gauge specific IgG antibody responses in sera of TB patients from Africa and South America. Open source software for general access to this method is provided.
Results: The IgG response pattern of TB patients differs from that of healthy individuals, with the molecular complexity of the antigens influencing the strength of recognition. South American individuals with or without TB exhibited a generally stronger serum IgG response to the tested *M. tuberculosis* antigens compared to their African counterparts. Individual *M. tuberculosis* peptide targets were defined, segregating patients with TB from Africa versus those from South America.
Conclusions: These data reveal the heterogeneity of epitope-dependent humoral immune responses in TB patients, partly due to geographical setting. These findings expose a new avenue for mining clinically meaningful vaccine targets, diagnostic tools, and the development of immunotherapeutics in TB disease management or prevention.

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Introduction

The quest to discover novel antigenic targets for the differential diagnosis of active and latent tuberculosis (TB) presents a challenging task. Although efforts are manifold and performed on a global scale, biological markers derived from *Mycobacterium tuberculosis* that can precisely distinguish between the various stages of *M. tuberculosis* infection and disease remain elusive.[1] Several field-based studies published in recent years have indeed provided valuable information while enriching the knowledge base pertinent to TB immunodiagnostics and clinically relevant immune responses.[2,3] Nevertheless, predominant factors, such as geographical setting of the study locations, socio-economic status of the study populations, and risk and rate of exposure to various strains of *M. tuberculosis* therein, in addition to the intricate biology of the pathogen itself, inevitably represent confounding factors.[4] In this regard, multiplatform analysis of immune responses in patients with TB, as well as individuals harbouring latent TB infection (LTBI), has initiated great interest due to the wealth of information made accessible to the global TB community.[1]

Identifying specific *M. tuberculosis*-derived antigenic determinants of adaptive immune responses (orchestrated by T- and B-cells), especially in individuals with LTBI, is clinically relevant since it will impact on patient treatment regimens. Due to the immense volume of data that is subsequently generated, stringent quality control measures are required in order to obtain a more true-to-life picture of pertinent immune responses.

M. tuberculosis epitope mining of 12-mer linear peptides derived from *M. tuberculosis* antigens, displayed on a microarray slide, has been reported previously.[5] This technology is based on the recognition and binding of antigen-specific human serum IgG to the respective cognate epitope. Differential antigen recognition patterns were observed between patients with pulmonary TB and healthy individuals from Armenia.[5] This method was further validated using soluble human leukocyte antigen (HLA) class II molecules, which could form complexes after binding to their respective peptides (derived from 61 different *M. tuberculosis* proteins) on the slide, thus providing additional information on the allelic restrictions for HLA-based presentation of immunogenic *M. tuberculosis* epitopes to CD4+ T-cells.[6] Three *M. tuberculosis* proteins first discovered to be immunologically relevant using this platform were evaluated among Honduran TB patients and healthcare workers exposed to *M. tuberculosis*,[7] as well as Belarussian patients with pulmonary TB.[8] The present study group is currently evaluating all three proteins as vaccine candidates in preclinical studies.

In this study, the serum antibody responses of patients with pulmonary TB from two different geographical regions with a high disease burden (Africa and South America) were evaluated using the peptide microarray platform. The recognition of specific *M. tuberculosis* epitopes in patients with active TB (TB+), as well as those without clinical disease (TB−), was assessed to generate an overall humoral immune response landscape. This information allowed the exact epitopes of immunogenic *M. tuberculosis* antigens that elicit measurable immune responses to be uncovered for the first time, and furthermore, the pattern of this response in health and disease, using immune recognition surfaces, by describing the chemical composition of antibody recognition independent of the biological nature of the respective *M. tuberculosis* antigen.

Materials and methods

Study subjects

Serum samples were provided by the Public Health Research Institute (PHRI), New Jersey, USA, and stored at −70 °C. Volunteers (TB patients and healthy individuals) classified under two geographically distinct cohorts were involved in this study: Africa ($n = 228$) and South America ($n = 139$). After matching by age and sex, only 120 patients (30 individuals per cohort) were included in the final analysis. The cohort description is provided in Table 1.

Slide production, scanning, and analysis

The peptide arrays were custom-manufactured by JPT (Berlin, Germany) as reported previously.[9] Slides consist of three identical sub-arrays, each with 6720 spots arranged in 16 blocks of 420 spots. Of the 6720 spots, 485 are negative 'empty' control spots, 271 are positive control spots (144 peptide controls, as described in Ngo et al.[10]), and 5694 are unique peptides generated from 154 *M. tuberculosis* proteins (listed in the **Supplementary Material**, Table S3) as 15-mers overlapping by five amino acid residues.

Sera were diluted 1:100 in 300 μl buffer (phosphate buffered saline (PBS), 3% foetal calf serum (FCS), 0.5% Tween 80; Sigma Aldrich, St Louis, MO, USA) and added to microarray chips for 16 h of incubation in a humid chamber at +4 °C. The slides were then washed with buffer twice and sterile distilled water three times. This was followed by secondary incubation with 300 μl diluted (1:500) Cy5-labelled mouse anti-human IgG monoclonal antibody IgG secondary antibody (catalogue number 6561-100; Abcam, UK) for 1 h at room temperature (RT) and then washing as before. The slides were then spun dry in a slide centrifuge (DJB Labcare, Newport Pagnell, UK).

Table 1
Description of the study cohorts from Africa and South America.

Continent	TB status	Number	Mean age (SD)	M:F ratio
Africa	Positive	89	37.6 (±15.2)	2.0
	Negative	140	40.2 (±16.2)	0.8
Total		228	39.2 (±15.8)	1.1
South America	Positive	72	33.9 (±12.7)	1.2
	Negative	67	41.5 (±15.6)	0.6
Total		139	37.6 (±14.6)	0.9

TB, tuberculosis; SD, standard deviation; M, male; F, female.