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IFN-γ, but not IP-10, MCP-2 or IL-2 response to RD1 selected peptides associates to active tuberculosis

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Summary *Objectives:* To evaluate whether *in vitro* response to *Mycobacterium tuberculosis* RD1 peptides selected by computational analysis, measured by IFN-γ, IP-10, MCP-2 or IL-2 production, is associated with active tuberculosis (TB) in a country with a high incidence of TB.
Methods: 129 individuals were prospectively enrolled, 41 with active-pulmonary TB and 88 without (household contacts and community controls). A whole blood assay based on RD1 selected peptides was performed. Soluble factors were evaluated by ELISA in plasma harvested at day1-post-culture. Enrolled individuals were also tested by QuantiFERON TB-Gold In tube (QFT-IT) and tuberculin skin tests (TST).
Results: IFN-γ response to RD1 selected peptides was significantly higher in active TB patients than in household contacts and community controls. IP-10 and MCP-2 response did not differ between active TB patients and household contacts, although it was higher in these groups compared to community controls; conversely IL-2 response did not differ among the three groups. When IFN-γ response to RD1 selected peptides was scored based on receiver-operator-characteristic analysis, active TB was predicted with 68% sensitivity and 86% specificity.

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QFT-IT and TST showed a sensitivity for active TB of 90% and 68% and a specificity of 58% and 59%, respectively.
Conclusions: IFN-γ (but not IP-10, MCP-2 and IL-2) response to RD1 selected peptides is associated with active TB with a higher specificity than QFT-IT and TST.

Background

A recent breakthrough in diagnosing *Mycobacterium tuberculosis* infection has been the development of T-cell-based Interferon Gamma Release Assays (IGRA) that use antigens belonging to *M. tuberculosis* region of difference (RD1), including early secreted antigenic target-6 [ESAT-6] and culture filtrate protein 10 [CFP-10].[1] Two commercial IGRA, based on overlapping peptides from CFP-10 and ESAT-6 are now available: QuantiFERON TB-GOLD In-Tube® (QFT-IT) (Cellestis Ltd., Carnegie, Australia) and T-SPOT.*TB*® (Oxford Immunotec, Abingdon, UK). Evidence reviewed elsewhere,[1–4] suggests that they are more specific than the tuberculin skin test (TST), and correlate better with markers of TB infection in low incidence settings. Importantly, the IGRA are less affected by bacillus Calmette–Guérin (BCG) vaccination than the TST.[1,4]

On the basis of this line of research, we recently reported an *in vitro* IFN-γ immune diagnostic assay for active TB, the novelty of which consists in the use of multiepitopic RD1 peptides that are selected by computational analysis.[5–11] The response to these peptides can be detected in subjects with ongoing *M. tuberculosis* replication, such as during active TB disease and/or recent infection, and decreases during TB therapy.[7,10,11]

Studies on IFN-γ response to the RD1 selected peptides conducted in countries with a relatively low incidence of TB (less than 10/100,000 population) such as Italy, Germany, Spain, and Bulgaria (less than 40/100,000 population),[12] suggest that the response to this assay is associated with active TB. Similar results were obtained in Uganda,[9] an African country with a high incidence of TB (incidence in 2004, 175/100,000). However, these studies showed that the diagnostic accuracy of this experimental assay needs to be improved before it can be proposed for clinical use.

It has been recently shown that the accuracy of IGRA may potentially be enhanced by the addition of other *M. tuberculosis*-specific antigens[13,14] by modifying the incubation step[15–17] or by measuring alternative/additional biomarkers. In this context it has been demonstrated that IFN-γ-inducible protein 10 (IP-10), monocytes chemotactic protein 2 (MCP-2) and interleukin (IL)-2 are additional biomarkers for LTBI detection after RD1-specific stimulation in both adults[18–24] and children.[25] IP-10 and MCP-2 are pro-inflammatory chemokines that are expressed in inflamed tissues by resident and infiltrated cells (primarily monocytes/macrophages) after paracrine stimulation from T-cells by interferon and other pro-inflammatory cytokines, or through innate mechanisms upon contact with viral, bacterial and fungal agents.[26–28] IP-10 is involved in trafficking monocytes and activated Th1 cells to inflamed foci.[26] Serum and pleural fluid IP-10 levels have been evaluated as biomarkers for diagnosis, prognosis, and monitoring of treatment efficacy in inflammatory and infectious diseases including TB.[29,30] IL-2 promotes T-cell replication and is essential for cellular immunity and granuloma formation. *M. tuberculosis* antigen-dependent IL-2 production has been demonstrated in patients with active TB[19–21] and its serum concentrations are elevated in patients with active TB, returning to normal with treatment.[31]

Thus, the objective of this study was to evaluate the assay based on the RD1 selected peptides in India. It is the country with the highest TB burden in the world and accounts for one fifth of the global incidence with an estimated 1.9 million cases annually.[32] In particular, our aims were to assess: i) whether IFN-γ response may help provide evidence of active TB; ii) whether biomarkers other than IFN-γ, such as IP-10, MCP-2 and IL-2, can be additional markers of specific TB responses. All enrolled individuals were also tested by QFT-IT and a fraction of them by TST.

Materials and methods

Population of patients

Study subjects were prospectively recruited during April 2007 and March 2008 in India at: i) Tuberculosis Research Centre, Chetput, Chennai and ii) P.D.Hinduja National Hospital and Research Center, Mumbai. The study was approved by the ethical committees of the two centers. Medical information and heparinised blood were obtained from subjects at enrolment after signing an informed consent.

The demographic details and information on previous tuberculin skin test (TST) results were collected. Individuals with a previous history of TB, Human Immunodeficiency Virus (HIV) infection, silicosis, in the final stage of renal disease, leukaemia/lymphoma, who had a TST in the past 16 months or had undergone anti-TB therapy or immunosuppressive therapy for more than two weeks, were excluded from the study. Pregnant and lactating patients were also excluded.

Active TB was defined as microbiologically confirmed if a sputum smear was positive for acid-fast bacilli on microscopy by Ziehl–Neelsen method and/or *M. tuberculosis* was identified in sputum culture in conventional Lowenstein Jensen (BioMérieux Inc., Marcy l'Etoile, France) and/or in liquid BacT/ALERT MP medium (BioMérieux Inc., Marcy l'Etoile, France). Patients with negative sputum smear and culture for *M. tuberculosis* were classified as having "clinical TB" if they had clinical, pathological and radiological findings consistent with tuberculosis, if an alternative diagnosis was excluded, and a full course anti-tuberculosis treatment was started with an appropriate clinical response.

Those without active TB were classified as healthy donors. They were free of TB symptoms and TB disease was ruled out by a chest X-ray and clinical examination. Those defined as "community controls", were recruited from families without