Title of the study: Identify immunologic markers of persons at highest risk of Progression of latent tuberculosis infection to tuberculosis

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Overall goal of the project. The goal of the project is to find novel biomarkers that will identify persons with latent tuberculosis infection (LTBI) who are at increased risk for progression to active tuberculosis (TB). We are studying household contacts of TB patients because the risk for developing TB is highest during the two years after infection, allowing identification of the maximum number of patients who will develop TB during the study period. The proposed study is to follow 1000 household contacts of TB patients for two years and drawing blood every 4 months to evaluate immune profile. Our primary goal is to find novel biomarkers that will identify persons with latent tuberculosis infection (LTBI) who are at increased risk for progression to active tuberculosis (TB), which is an objective of this Indo-US collaborative program.

Using biomarkers to identify persons with LTBI who are at great risk for development of TB would allow focused application of isoniazid treatment to a relatively small population. For example, if we find that 20% of contacts with LTBI have biomarkers associated with a 25% risk of developing TB in 2 years, whereas TB develops in <1% of those without these biomarkers, this reduces the number of persons requiring isoniazid by 80% and makes more resources available to ensure that the 20% of high-risk contacts with LTBI complete directly observed preventive therapy. While most studies of biomarkers of TB have focused on T-cells and cytokines, our preliminary and published data suggest that macrophages, natural killer (NK) cells and T regulatory cells (Tregs) contribute to human immunity against TB. Therefore, we will determine if biomarkers based on these cell populations predict development of TB in

1,000 persons with LTBI, though three specific aims.

The specific aims of the study are:

Aim 1. Determine if monocyte phenotype and function predict development of TB.
Aim 2. Determine if NK cell phenotype and function predict development of TB.
Aim 3. Determine if T-regulatory cell numbers, immunosuppressive function and antimicrobial activity predict development of TB.

Study population:

Longitudinal cohort description:

City and population. The proposed studies will be performed in Hyderabad, which is the capital of the state, Andhra Pradesh, with a population of about 80 million. It is one of India's states with a high prevalence of HIV/AIDS. The greater Hyderabad urban area is one of the fastest growing places in India. It is the fifth largest city in the country, with a population of over 5.5 million and an area of 236 sq. kms. It is estimated to have the fifth largest slum population in the country, with the number of notified slums increasing phenomenally from 106 in 1962 to 841 in 2001, with many slums both in the inner city as well as in the suburbs. Almost all the people living in the slums are either daily wage earners such as house building construction workers, street vendors or unemployed persons. There are equally large numbers of people who live on the fringes of the slums and whose economic status is only marginally better, making them wholly dependent on state facilities. The rapidly growing slum and non-slum population is a serious challenge for public health.

Description of the proposed research sites.

Blood donor recruitment will takes place at two institutions. 1. Bhagawan Mahavir Hospital and Research Centre (BMHRC). 2. The Blue Peter Public Health & Research Center (BPHRC). Both institutes are located 30 miles apart.

Tuberculosis Program at Bhagawan Mahavir Hospital and Research Centre (BMHRC). BMHRC is a non-profit trust hospital that initiated a program in 1995 under the Revised National TB Control Programme, to determine if private practitioners and the government can collaborate to implement directly observed therapy (DOTS) effectively. BMMRC established a Public-Private Mix – DOTS programme in 1995. All the 110 private practitioners in the area were encouraged to refer symptomatic patients to the hospital for sputum examination and DOTS. The study in the first 6 years was supported by the Department for International Development and sponsored by WHO, Geneva. Initially this program covered a population of 100,000 and later, this was expanded to 500,000. In 2004, BMHRC was chosen by the Indian government to carry out the Global Fund for AIDS, Tuberculosis and Malaria Project of Urban DOTS in Hyderabad. The project has been operational since February 1st, 2005. Furthermore, BMHRC is part of the Centre of Excellence for Tuberculosis, supported by DBT titled, “Multidisciplinary approaches aimed at interventions against Mycobacterium tuberculosis”. The late Dr. KJR Murthy was instrumental in establishing these programs, and was honored by former President William J Clinton of the USA, who visited Mahavir Hospital on World TB Day in 2000. The Indian PI in the current proposal, Dr. Valluri, and the late. Dr. Murthy worked together for more 20 years. Currently Dr. Valluri is principal investigator at the Blue Peter Public Health & Research Center (BPHRC). Over the past decade, this team has followed hundreds of TB patients to determine their clinical course and outcome, and large numbers of household contacts for development of TB. They are experienced in collecting blood samples to obtain plasma, serum and peripheral blood mononuclear cells for immunologic assays and for extraction of DNA and RNA.

Tuberculosis Program at The Blue Peter Public Health & Research Center (BPHRC). BPHRC is associated with the LEPRA society a non-governmental organization providing community based services in TB and other infections in the states of Andhra Pradesh and Orissa, and conducting applied research in these infections. The LEPRA society has partnered with the Revised National TB control Programme since 1998, serving a population of approximately 6,000,000 in Hyderabad and the surrounding areas. LEPRA participates in TB control through direct delivery of patient services, capacity building, advocacy, communication and most importantly, field research. This gives LEPRA a strong presence in both TB and HIV control programmes.

Inclusion and exclusion criteria:

To be considered eligible for enrollment, the participant must meet the following criteria:

1. Is an adult or child with exposure to an adult with untreated or inadequately treated pulmonary TB.
2. Has no clinical signs or symptoms of active TB or history of active TB.
3. Has proved a written informed prior to his/her first sample or other study-specific data being collected.
4. Agrees to the collection and storage of specimens for use for future research.

Exclusion Criteria

Following are the exclusion criteria for the study:

1. Pregnancy.
2. HIV infection.
3. Age below 6 and above 60 yrs.
4. Plans to move out of study area.
5. Does not provide consent to participate.

STUDY DESIGN, METHODS, CLINICAL AND LABORATORY EVALUATIONS AND TIMELINES

We will study household contacts of TB patients because the risk for developing TB is highest during the two years after infection, allowing identification of the maximum number of patients who will develop TB during the study period. Among household contacts of TB patients, approximately one-third become infected with *M. tb,* as indicated by a positive tuberculin skin test. Approximately 4% of these tuberculin positive household contacts develop TB over 2 years. Household contacts with LTBI in the United States are targeted for isoniazid treatment to prevent TB, but current medical practice in India and most developing nations is not to treat these individuals because limited public health resources are focused on treatment of patients with active TB.

Study population. We will study 1000 household contacts of TB patients diagnosed in Hyderabad, India, through our collaborators, Dr. Valluri, at the Blue Peter Hospital Research Center-Lepra Society (BPHRC), and at the Bhagawan Mahavir Medical Research Center at Mahavir Hospital**.** The clinic at Mahavir Hospital is a national center for treatment of TB that covers a population of 500,000, living in twenty localities are covered with five microscopic centres**.** Half of the population living in slums where the prevalence of TB is extremely high. Mahavir Hospital has run a directly observed therapy-short course (DOTS) program since 1995 and the personnel have extensive experience in following TB patients throughout the course of therapy. The median number of household contacts per patient was 6. Of these, approximately one-fifth are <18 years old. In last 5 years (2006-11), a total number of 5307 patients were

diagnosed with tuberculosis. Of them, 2046 (39%) had pulmonary TB with sputum positive by microscopy. 12 –15 % of the patients (index) who have been treated at the clinic and considered cured, transmit the disease to other household members or their neighbours (contacts). Dr. Valluri has on-going projects funded by the Indian Council of Medical Research and Department of Biotechnology, for which they established a TB patient household contact cohort of 2000 subjects, collecting serum and DNA samples for gene polymorphism studies. BPHRC runs six out-patient clinics, three in-patient wards with 70 beds, six laboratories and 21 microscopy centres. A population of about 5,21,000 living in twenty localities are covered with five microscopic centres (Gaddiannaram, Seethaphalmandi, Dhoolpet, Bhavaninagar, King Koti). During the study period, we will recruit 1,000 household contacts, aged 6 years or older.

Initial screening tests. All donors will be screened for HIV infection by ELISA. HIV-negative persons will be selected, and a QuantiFERON-TB Gold (QFN) test will be performed. Because BCG vaccination is routine in India, ***QFN will be used instead of the tuberculin skin test because it is more specific for LTBI in BCG vaccinated persons***. The QFN test is based on the T-cell response to *M. tb*-specific proteins that are not shared by BCG. If the initial QFN test is negative, another test will be performed 4 months later. QFN+ persons will be evaluated for TB by chest radiograph and clinical evaluation. If they have no evidence of TB, they will be considered to have LTBI. Subjects with two negative QFN tests will be considered to be uninfected with *M. tb.* Because the baseline prevalence of LTBI in Hyderabad is estimated to be approximately 60%, we anticipate that two-thirds of the 1,000 household contacts will have LTBI. Demographics, medical history, and clinical data will be collected. CBC and lymphocyte count will be determined.

Follow-up of study subjects. We will try to recruit maximum number of donors in the first year. All persons with LTBI will be evaluated every 4 months for two years. At each visit, subjects will be evaluated for TB, and blood samples of 10-40 ml will be obtained, depending on their age and weight. We anticipate loss of approximately 150 persons (15%) during follow-up. From this population, we expect that 20-32 persons will develop TB. All subjects with symptoms of possible TB will be referred for clinical evaluation and treatment. Subjects with: 1) clinical specimens that show acid-fast bacilli on smear or *M. tb* on culture; or 2) chest radiographic changes consistent with TB and a clinical response to antituberculosis therapy, will be classified as having TB.

Data Collection

The team will be responsible for the development of case report forms (CRFs) needed to collect the information required to implement this study. Study monitoring data, including information about eligibility, demographic data, and medical history will be collected on CRFs. All participant-related study information will be identified through the PID on all CRFs. Names and other personal identifiers will not be used on any CRFs, study-specific laboratory specimens, clinical evaluations, or laboratory results. The source documents, and CRFs will be maintained at the study sites.

Statistical considerations, sample size calculations and plans for data analysis

For the power calculations below, we assume that data will be normally distributed among those with LTBI and those who develop TB. If this is not the case, comparisons between groups will be done by the non-parametric Wilcoxon rank-sum test. We provide examples below for analysis of one variable for each of the three specific aims. For all variables, we assume that findings in persons with LTBI who do not develop TB will be similar to those of normal individuals. For aim 1, based on our published data (1), we estimate that, in persons with LTBI who do not develop TB, that 21 ± 7%

of PBMC will be CD14hiCD16-. With estimated sample sizes of 64 persons who develop TB patients and 736 persons with LTBI who will not, we can detect an increase in the percentage of CD14hiCD16- cells of 2.5% with an α of 0.05 and a power of 80%. For aim 2, based on our published data, IL-22 levels produced by NK cells in persons with LTBI who do not develop TB should be 420 +/- 100 pg/ml. If 64 persons develop TB and 736 do not, we can detect a mean decrease in IL-22 levels of 37 pg/ml with an α of 0.05 and a power of 80%. For aim 3, based on our published data, the frequency of TGF-β+ CD4+CD25+FoxP3+ cells is estimated to be 66 +/- 8 pg/ml. With 64 persons who develop TB and 736 who do not, we can detect a mean increase of 3 pg/ml in TGF-β levels, with an α of 0.05 and a power of 80%.

TIMELINE

We submitted this proposal for 5 year funding (fCRU) and the application was selected for 3 year funding (dCRU). We will try to recruit maximum number of donors in the first year of the study. Blood will be drawn from the donors every four months and follow-up will be completed for all patients by the end of year 3. During the last 3 months of study, we will complete data analysis and continue to perform confirmatory and additional experiments with frozen cells and RNA.