

Summary

Section 1 – Project objectives and major achievements during the reporting period

The main goals of this project are 1) to organize the collect, storage and analysis of a large collection of *Mycobacterium tuberculosis* strains (6000) from the 31 Provinces of China, and of information on TB patients who provided samples, 2) to create a database and perform epidemiological studies to identify the origin of the Beijing lineage . The data will be compared to the characteristics of strains collected in other countries. This will produce knowledge on the diversity of Chinese TB strains and show whether the dispersion of the Beijing type is of clonal origin.

The present SSA aims at preparing the project in collaboration with Chinese scientists headed by Pr. Kanglin Wan, transfer the technical knowledge, follow the progress of the experiments, collect the data in a shared-database and produce population analyses.

During the first year we have defined the characteristics of the patients to be included in the protocol and we have chosen the appropriate methods for collecting and storing strains, for genotyping and multidrug-resistance (MDR) assessment. These tasks were performed in close collaboration by the teams in Orsay (Christine Pourcel co-ordinator of the SSA and Gilles Vergnaud) and in Bilthoven (Dick van Soolingen, partner 2 and Kristin Kremer). As a pilot assay we have concentrated our work on TB strains isolated in the Beijing Province. Data on the BCG status, age and gender of the patients as well as other relevant clinical information have been collected. Technical knowledge has been transmitted to the Chinese CDC in Beijing.

Section 2 – Workpackage progress of the period

WP1: Objectives

Defining the **patient cohort**, collect the samples to isolate *M. tuberculosis* strains, and characterize the strain phenotype (MDR).

Project leader: Wan Kanglin

Progress toward objectives

During the first meeting held in Orsay in may 2006, the partners decided on the number and characteristics of patients to be included in the study. A repartition according to age, gender and BCG status will be made. The format of the database was discussed. We favour the use of Bionumerics, a multipurpose software for Image analyses and database management.

Pr Kanglin Wan together with Heads of health centres in all the Chinese provinces, is organising the collect of specimens from which *M. tuberculosis* will be cultured. Different documents are gathered: patient inform consent and subject information sheet, questionnaire and Table of Examination of Patient and Samples Preservation. A precise identification tag is given to each new strain. All the strains included in the program are sent to Beijing and their characteristics are entered in the database. The CDC in China is responsible for checking the antibiotic resistance profile of the strains and for storing them alive in a secured -80°C freezer.

At the present time 3763 samples and patient information forms have been collected in the Beijing province and DNA was prepared for genotyping . The patient are distributed into four age groups: older than 60, between 60 and 30, between 30 and 18 years old and younger than 18 years, with an equal number of males and females. The major problem is in collecting strains from the youngest age group ie. less than 18 years old which will delay this phase of the project.

As planed in the present SSA only the data for 103 patients of the Beijing province have been entered in the database. The remaining data will be stored in a computerised fashion when approval will be obtained from ethical committees.

WP2: Objectives

Different genotyping techniques will be applied to the TB strains including spoligotyping, VNTR typing (MLVA16), analysis of deletions. Whenever possible we wish to teach people in local health centres to perform **genotyping** studies; although this will not be possible in some centers due to lack of expertise and equipment.

The different steps include: training of Chinese scientists in Orsay and Bilthoven, transfer of technical knowledge to Chinese laboratories and validation of techniques, follow-up of large-scale genotyping, analysis of images, centralisation of data and production of dendrograms.

Project leader: Christine Pourcel

Progress toward objectives

During a 6 months stay in Orsay, and a shorter stay in Bilthoven, Jinghua Liu has been trained to perform different molecular techniques to genotype the TB isolates. She has analysed 103 isolates from the Beijing province and found that 91.3% belong to the Beijing family as previously observed on smaller series. She is in charge of transferring the techniques to the CDC in Beijing and to ensure that each participating laboratory masters the procedure through

quality assays and controls. She has also attended a workshop on the Bionumerics software in Ghent, Belgium.

Precise protocols to perform the analyses have been prepared in order that the data can be added to the database in the correct format. In November 2006 the different partners participated in a meeting in Beijing, in which attendants from 15 provinces came to learn how to perform genotyping for epidemiological studies.

We are preparing a set of 20 samples corresponding to strains previously typed in Orsay and belonging to diverse genetic group, to be distributed to participating laboratories in China, together with the primers to be used in PCR reactions and molecular weight controls.

These details can be found on the web site dedicated to this project.