CHAPTER 1

Review of Literature

1.1. Genus *Mycobacterium* 1
   1.1.1. Classification 1
   1.1.2. Features of mycobacteria 1
   1.1.3. Unique cell envelope of mycobacteria 1
   1.1.4. Proteome of the cell membrane of *M. tuberculosis* 2

1.2. Mycobacterium *tuberculosis* and tuberculosis 3
   1.2.1. Epidemiology of tuberculosis 3
   1.2.2. Pathogenesis 5
      1.2.2.1. Infection and granuloma formation 5
      1.2.2.2. Immune response and the outcome of infection 6
   1.2.3. Control measures 8
   1.2.4. Vaccines: BCG as a vaccine 9
   1.2.5. Diagnosis of tuberculosis 10
      1.2.5.1. AFB staining 10
      1.2.5.2. Culture 10
      1.2.5.3. Mantoux test-tuberculin skin testing 11
      1.2.5.4. Molecular methods 12
      1.2.5.5. Tests based on secretory proteins 12

1.3. Culture filtrate proteins (CFPs) 14
1.3.1. Excretory - secretory antigens  
1.3.2. Secretory proteome of *M. tuberculosis*  
1.3.3. CFPs as vaccine candidates  
1.3.4. Mechanism of secretion of mycobacterial CFPs  
1.3.5. CFPs of vaccine/diagnostic potential  
  1.3.5.1. Heat shock proteins and Ag85 complex  
  1.3.5.2. ESAT family  
1.3.6. Commercial tests based on CFPs  
  1.3.6.1. Interferon-gamma (IFN-γ) tests  
  1.3.6.2. Tests based on the detection of antibodies against CFPs  

1.4. Iron deprivation in mycobacteria  
  1.4.1. Role of iron in bacteria  
  1.4.2. Adaptation of bacteria to conditions of iron limitation  
  1.4.3. Iron acquisition in mycobacteria  
    1.4.3.1. Mycobactins  
    1.4.3.2. Carboxymycobactins  
    1.4.3.3. Exochelins  
    1.4.3.4. Identification of IREPs in Mycobacteria  
  1.4.4. Global response of *M. tuberculosis* to iron availability  
  1.4.5. Iron-dependent regulation at molecular level  
  1.4.6. Iron and virulence factors  

**Objectives of the study**  

**CHAPTER 2**  

**Materials and Methods**  

2.1.1. Sources of chemicals  
2.1.2. Bacterial strains  
2.2.1. Growth of *M. tuberculosis* and BCG strains under high and low iron conditions  
2.2.2. Growth of *M. tuberculosis* with varying concentrations of iron
2.2.3. Time-dependant growth of *M. tuberculosis* 34

2.3. Assay of mycobactin and carboxymycobactin 34
   2.3.1. Assay of carboxymycobactin by Universal CAS assay 35
   2.3.2. Assay of mycobactin 35

2.4. Analysis of culture filtrate proteins (CFPs) 36
   2.4.1. Concentration of CFPs by ammonium sulphate precipitation 36

2.5.1. Separation of CFPs 36
   2.5.2. Tris-Tricine gel electrophoresis for the separation of low molecular weight proteins 38
   2.5.3. Two-dimensional polyacrylamide gel electrophoresis: 2D – PAGE 39
      2.5.3.1. Sample preparation 39
      2.5.3.2. Rehydration 39
      2.5.3.3. First dimension separation (IEF) 39
      2.5.3.4. Second dimension separation using SDS-PAGE 40
      2.5.3.5. Silver Staining 40
      2.5.3.6. Fast Coomassie Brilliant Blue staining 41
      2.5.3.7. Scanning and image analysis of the 2D-gel proteins 42

2.6. Identification of CFPs by MALDI-TOF MS analysis 42
   2.6.1. Processing of 2D PAGE spots for MALDI-TOF MS analysis 42
   2.6.2. Western blotting analysis of CFPs 43

2.7. CF-Ag pools: preparative gel electrophoresis 43
   2.7.1. Immune proliferation studies with CF-Ag pools 44
      2.7.1.1. Preparation of RPMI media 44
      2.7.1.2. Study group: selection of tuberculosis patients and normal control individuals 45
      2.7.1.3. Isolation of lymphocytes from blood 46
      2.7.1.4. Lymphocyte proliferation assay 46
   2.7.2. Assay of interferon – γ in the culture supernatants of stimulated T cells 47
CHAPTER 3

Results

3.1. Iron levels in *Mycobacterium tuberculosis*  
3.1.1. Effect of iron levels on mycobactin and carboxymycobactin  
3.1.1.1. Establishment of growth of *M. tuberculosis* under high and low iron conditions  
3.1.1.2. Iron-dependant expression of mycobactin and carboxymycobactin  
3.1.2. Effect of iron levels on the expression of culture filtrate proteins (CFPs)  
3.1.3. Time-course studies  
3.1.3.1. Time-course expression of mycobactin and carboxymycobactin  
3.1.3.2. Time-course expression of CFPs  
3.1.4. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of CFPs  
3.1.4.1. MALDI-TOF analysis of CFPs  
3.1.4.2. Identification of CFPs by co-migrational analysis and immunoblotting with anti-CFP antibodies  
3.1.4.3. Identification of ESAT-6 proteins  
3.2. Iron levels in *M. bovis* BCG strains  
3.3. Gel elution and preparation of culture filtrate antigen pools  
3.3.1. Preparative SDS-PAGE and gel elution of CF-Ag pools  
3.3.2. 2D-PAGE of the CF-Ag pools  
3.4. Immune response studies with iron-regulated culture filtrate antigens
3.4.1. Immune response of PBMCs to whole CFPs and PPD

3.4.2. Immune response studies with defined CF-Ag pools
   3.4.2.1. Lymphocyte response of normal healthy individuals
   3.4.2.2. Lymphocyte response of smear positive group of tuberculosis patients
   3.4.2.3. Lymphocyte response of smear negative group of tuberculosis patients
   3.4.2.4. Lymphocyte response of extra pulmonary tuberculosis patients

3.4.3. Expression of IFN-γ: stimulation with whole CFPs and PPD

3.4.4. Expression of IFN-γ: stimulation with CF-Ag pools
   3.4.4.1. Normal healthy individuals
   3.4.4.2. Smear positive tuberculosis patients
   3.4.4.3. Smear negative tuberculosis patients
   3.4.4.4. Extra-pulmonary tuberculosis patients

3.4.5. Antibody-based detection of mycobacterial culture filtrate antigens
   3.4.5.1. Screening of serum samples using Mycotest
   3.4.5.2. ELISA
   3.4.5.3. Performance of Mycotest and ELISA

CHAPTER 4: Discussion

Summary

References