# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>I-III</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>IV</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>V-VIII</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>IX-XV</td>
</tr>
<tr>
<td><strong>CHAPTER ONE</strong></td>
<td>1-43</td>
</tr>
<tr>
<td><strong>REVIEW OF LITERATURE</strong></td>
<td></td>
</tr>
<tr>
<td>1.1. PATHOGENESIS OF TUBERCULOSIS</td>
<td></td>
</tr>
<tr>
<td>1.2. IMMUNE RESPONSE TO TUBERCULOSIS</td>
<td></td>
</tr>
<tr>
<td>1.3. ROLE OF CYTOKINES IN TUBERCULOSIS: A BALANCING ACT</td>
<td></td>
</tr>
<tr>
<td>1.4. GRANULOMA IN TUBERCULOSIS: BATTLE FIELD OR SHELTER FOR MYCOBACTERIA</td>
<td></td>
</tr>
<tr>
<td>1.5. SURVIVAL OF MYCOBACTERIA: DITCHING THE HOST IMMUNE RESPONSE</td>
<td></td>
</tr>
<tr>
<td>1.6. ROLE OF HLA IN CELL MEDIATED IMMUNITY</td>
<td></td>
</tr>
<tr>
<td>1.7. DEFINITION OF THE PROBLEM</td>
<td></td>
</tr>
<tr>
<td><strong>CHAPTER TWO</strong></td>
<td>44-85</td>
</tr>
<tr>
<td>IMMUNE RESPONSE IN TUBERCULOSIS PATIENTS, HOUSEHOLD CONTACTS AND HEALTHY CONTROLS</td>
<td></td>
</tr>
<tr>
<td>2.1. INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>2.1.1 Regulatory T cells</td>
<td></td>
</tr>
</tbody>
</table>
2.1.2 Immunosuppressive functions of Tregs

2.1.3 Role of Tregs in tuberculosis: Friend turning foe

2.2. MATERIALS AND METHODS

2.2.1 Study population

2.2.2. Recombinant antigen and peptide

2.2.3. Prediction of epitopes of CFP-10 using Propred I and CTLpred bioinformatic tools

2.2.4. Isolation of PBMCs

2.2.5. RNA isolation

2.2.6 cDNA synthesis

2.2.7 Reverse transcriptase PCR (RT-PCR)

2.2.8 Cytokine mRNA analysis of unstimulated PBMCs

2.2.9 Cytokine mRNA analysis of stimulated PBMCs

2.2.10 ELISPOT assay

2.3. RESULTS

2.3.1. Cloning, expression and purification of Ag85A

2.3.2. Peptide prediction of CFP-10 peptide

2.3.3. Analysis of FoxP3 and cytokine levels in unstimulated PBMCs

2.3.3.1 Cytokine and FoxP3 mRNA analysis

2.3.3.2 Increased levels of FoxP3 and TGF-β of unstimulated PBMCs differentiate patients from contacts

2.3.4. FoxP3 and cytokine mRNA analysis of stimulated PBMCs

2.3.4.1 Cytokine and FoxP3 mRNA analysis

2.3.4.2 Increased FoxP3, IL-10 and TGF-β in patients following stimulation of PBMC’s
2.3.5 IFN-γ ELISpot assay

2.4. DISCUSSION

CHAPTER THREE

MONOCYTES OF TUBERCULOSIS PATIENTS AND HEALTHY CONTROLS

3.1. INTRODUCTION

3.1.1 Role of cytokines in granuloma

3.1.2 Multinucleate giant cells

3.1.3 Models of granuloma study

3.2. MATERIALS AND METHODS

3.2.1. Study population

3.2.2. In vitro MGC formation

3.2.3. Cytokine ELISA

3.2.4. Effect of IL-10 and anti-IL-10 on MGC formation

3.2.5. Effect of IL-4 and anti-IL-4 on MGC formation

3.2.6 Cytokine analysis of culture supernatant of patients before and after treatment

3.3. RESULTS

3.3.1. In vitro MGC formation

3.3.2. Cytokine analysis of culture supernatant by ELISA

3.3.3. Role of IL-10 and IL-4 in in vitro MGC formation

3.3.4 Comparison of in vitro MGC formation in patients before and after treatment
3.3.5 Cytokine bead array analysis of patients' in vitro activated lymphocyte culture supernatant before and after treatment

3.4. DISCUSSION

CHAPTER FOUR

2D-GEL ELECTROPHORETIC ANALYSIS OF MONOCYTES FROM TUBERCULOSIS PATIENTS, HOUSEHOLD CONTACTS AND CONTROLS

4.1. INTRODUCTION

4.2. MATERIALS AND METHODS

4.2.1. Isolation of peripheral blood monocytes

4.2.2. Protein sample preparation from monocytes

4.2.3. Two-dimensional electrophoresis

4.2.4 Spot identification

4.2.5 Total RNA isolation and reverse transcription

4.2.6 Quantitative PCR

4.2.7 Western Blot Analysis

4.2.8 Monocyte adhesion assay

4.3. RESULTS

4.3.1 Differential protein expression in tuberculosis patients

4.3.2 Protein identification

4.3.3 qPCR analysis of αII-spectrin

4.3.4 Western Blot Analysis

4.3.5 Monocyte adhesion assay

4.4. DISCUSSION
CHAPTER FIVE

STUDY OF CYTOTOXIC T LYMPHOCYTES IN PATIENTS AND CONTROLS

5.1. INTRODUCTION

5.2. MATERIALS AND METHODS

5.2.1. In silico analysis of M. tuberculosis antigens with protective and susceptible HLA alleles

5.2.1.1 M. tuberculosis antigens analyzed

5.2.1.2 HLA alleles

5.2.1.3 Prediction of epitopes with CTLpred

5.2.2. Patient and Healthy controls

5.2.3. RNA isolation and cDNA synthesis

5.2.4. PCR amplification and identification of HLA-B allele

5.2.5. Prediction of epitopes with Propred I and CTLpred

5.2.6. Cell lines

5.2.7. Expansion and purification of effector T cell population

5.2.8 Cytotoxicity assay

5.2.9 Statistical analysis

5.3. RESULTS

5.3.1 In silico analysis of protective and pathologic tuberculosis antigens

5.3.2 HLA identification for various individuals included in the CTL study
5.3.3 Peptide prediction for HLA-B*4403 from various M. tuberculosis antigens

5.3.4 Cytotoxicity assay

5.4. DISCUSSION

REFERENCES 170-206
PUBLICATIONS 207