CONTENTS

Abstract i-ii
List of Abbreviations iii-iv
List of Tables v
List of Figures vi-viii
Contents ix-xvii

Chapter One

Review of Literature

Part A: What are vaccines? 1-4

1.1. Types of vaccines 1-2
   1.1.1. Inactivated or killed vaccine 1
   1.1.2. Attenuated vaccine 2
   1.1.3. Subunit vaccine 2

1.2. Other vaccine strategies 3-4
   1.2.1. Virus like particle vaccine 3
   1.2.2. Toxoid vaccine 3
   1.2.3. Conjugate vaccine 3
   1.2.4. Cell-based vaccine 3
   1.2.5. Recombinant vaccine 4
   1.2.6. DNA vaccine 4
   1.2.7. Peptide vaccine 4

Part B: Vaccines against intracellular pathogens 4-27

1.3. General Introduction 4-10
   1.3.1. Vaccines against intracellular pathogens 5-8
      need to elicit immune response different
      from that evoked by extracellular pathogens
   1.3.2. Different approaches to the development 8-10
      of vaccines for intracellular and extra cellular
      pathogens
   1.3.3. The identification of protective subunits for 10
intracellular pathogens remains a major challenge

1.4. Vaccines against *Candida albicans* 10-15

1.4.1. Driving forces in development of anti-fungal vaccines in general and anti-*Candida* vaccines in particular 11-12

1.4.2. Antigenic composition and formulation: the critical role of adjuvants 12-13

1.4.3. Vaccines against *C. albicans* in pre-clinical settings 13-15

1.4.4. Anti-*Candida* vaccines in clinical trial 15

1.5. Vaccines against *Mycobacterium tuberculosis* 16-27

1.5.1. TB infection control and *M. bovis* BCG: 18-20

The current TB vaccine

1.5.2. Designing better TB vaccines:

Two basic and complementary approaches

1.5.2.1. *Live vaccines, aiming to replace BCG as priming vaccines* 22-24

1.5.2.2. *Subunit vaccines, primarily aiming to boost rBCG-induced responses* 24-25

1.5.3. Designing better TB vaccines: the next generation

1.5.3.1. *New antigens* 25-26

1.5.3.2. *Therapeutic vaccines* 27

Part C: Nanoparticle based vaccines 27-41

1.6. Types of nanoparticles used in vaccine development

1.6.1. Polymeric nanoparticles 29-30

1.6.2. Inorganic or non-degradable nanoparticles 3-31

1.6.3. Liposomes 31-32

1.6.4. Immunostimulating complex (ISCOM) 32

1.6.5. Virus-like particles 32-33

1.6.6. Self-assembled proteins 33-34
Chapter Two

Amyloid form of Ovalbumin evokes native antigen specific immune response in the host: Prospective immunoprophylactic potential

2.1. Introduction 49-51

2.2. Materials and Methods 52-59

2.2.1. Chemicals and reagents 52
2.2.2. Fibril formation 52
2.2.3. Rayleigh Scattering measurements 52
2.2.4. Turbidity measurements 52
2.2.5. CR binding studies 53
2.2.6. ThT binding studies 53
2.2.7. CD measurements 53
2.2.8. Transmission Electron Microscopy 54
2.2.9. In vitro release kinetics 54
2.2.10. Animals 54
2.2.11. Ethics statement 54
2.2.12. Mode and schedule of immunization 55
2.2.13. Collection of sera 55
2.2.14. Determination of antigen specific total IgGs 55
   by ELISA
2.2.15. Determination of antibody isotypes 55
   in sera of immunized mice
2.2.16. Measurement of DTH response 56
2.2.17. Determination of NO production 56
2.2.18. T lymphocyte isolation from spleens of
   immunized mice
2.2.19. Lymphocyte proliferation assay 57
2.2.20. Cytokine assay: Determination of IFN-γ,
   IL-4 and IL-12 by sandwich ELISA
2.2.21. Dot blot assay 58
2.2.22. Western blot assay 58
2.2.23. Interaction of macrophages with
   amyloidal aggregates
2.2.24. Ovalbumin molecular modelling 59
2.2.25. Statistical analysis 59

2.3. Results 60-86

2.3.1. Continuous shaking over extended time 60
   period induces aggregation of soluble OVA
2.3.2. Extended incubation with shaking leads to
   the formation of β-sheet rich OVA aggregates
2.3.3. Aggregates formed at pH 7.0 have 67
   fibrillar while aggregate species generated
   at pH 2.5 have suprafibrillar morphology
2.3.4. The sturdy fibrillar amyloids release OVA 69
   in a sustained manner
2.3.5. The aggregates possibly release monomeric/
   multimeric forms of OVA 69-70
2.3.6. The released OVA seems to acquire native
   conformation and does not exhibit
general characteristics of amyloid aggregates

2.3.7. Antibodies developed upon administration of aggregated forms of OVA can recognise native antigen

2.3.8. OVA released by various forms of aggregates interacts with native OVA specific antibodies

2.3.9. OVA released by various forms of aggregates exhibits reactivity with anti-native OVA 2D11 monoclonal antibody

2.3.10. Macrophages phagocytose OVA aggregates

2.3.11. The OVA aggregates based immunization evokes Th1 cytokines in the host

2.3.12. Lymphocyte proliferative response

2.3.13. OVA aggregates evoke delayed type hypersensitivity in immunized animals

2.3.14. Nitric oxide production

2.4. Discussion

Chapter Three

MP65 amyloid fibril based vaccine imparts protection against experimental murine disseminated candidiasis

3. 1. Introduction

3.2. Materials and Methods

3.2.1. Chemicals and reagents

3.2.2. Purification of MP65

3.2.3. Fibril formation

3.2.4. Rayleigh scattering measurements

3.2.5. Turbidity measurements

3.2.6. ThT binding studies

3.2.7. CR binding studies

3.2.8. CD measurements

3.2.9. Transmission electron microscopy

3.2.10. Preparation of liposomes
3.2.11. In vitro release kinetics 103
3.2.12. Animals 103
3.2.13. Ethics statement 103
3.2.14. Mode and schedule of immunization 104
3.2.15. Collection of sera 104
3.2.16. Determination of MP65-specific total IgGs by ELISA 104
3.2.17. Determination of antibody isotypes in sera of immunized mice 105
3.2.18. Measurement of DTH response 105
3.2.19. Determination of NO production 105
3.2.20. T lymphocyte isolation from spleens of immunized mice 106
3.2.21. Lymphocyte proliferation assay 106
3.2.22. Determination of IFN-γ, IL-4 and IL-12 by sandwich ELISA 106
3.2.23. Staining of T cells for memory and macrophages for co-stimulatory markers 107
3.2.24. Dot blot assay 108
3.2.25. Western blot assay 108
3.2.26. Macrophage interaction with amyloids 108
3.2.27. Challenge of animals with *Candida albicans* infection 109
3.2.28. Determination of fungal load in various vital organs 109
3.2.29. Statistical analysis 109

3.3. Results 110-133

3.3.1. Characterization of purified MP65 for size and biochemical as well as immunological properties 110
3.3.2. Monitoring aggregation of soluble MP65 by light scattering and turbidity measurements 110
3.3.3. Analysing fibril formation by ThT 111
fluorescence measurements

3.3.4. Transmission electron microscopy

3.3.5. MP65 fibrils release monomers in a slow and sustained manner

3.3.6. CR binding

3.3.7. Far UV-CD spectra

3.3.8. MP65 released from its fibril exhibits biophysical characteristics of native MP65

3.3.9. Antibodies generated in response to MP65 fibril immunization recognise native antigen

3.3.10. Uptake of amyloidal MP65 aggregates by macrophages

3.3.11. Humoral response upon immunization with MP65 amyloid fibril

3.3.12. MP65 fibril induces Th1 cytokines in the host

3.3.13. MP65 fibril stimulates T cell proliferation

3.3.14. MP65 fibril immunization induces increased expression of co-stimulatory and memory markers as revealed by FACS analysis

3.3.15. MP65 fibril evokes delayed type hypersensitivity in immunised animals

3.3.16. Nitric oxide production

3.3.17. Protective efficacy of MP65 fibril against candidiasis

3.4. Discussion
Chapter Four

Co-administration of escheriosome entrapped TLR agonist with RD antigen based nonovaccine induces protective T cell memory response and imparts protection against experimental murine tuberculosis

4.1. Introduction 140-142

4.2. Materials and Methods 143-149

  4.2.1. Chemicals and Reagents 143
  4.2.2. *M. bovis* BCG and *M. tb* strain 143
  4.2.3. Cloning, expression and purification of Rv3620c 144
  4.2.4. Isolation of total polar lipids of Archaebacteria 144-145
  4.2.5. Isolation of *E. coli* lipids 145
  4.2.6. Preparation of liposome and assessment of antigen entrapment efficiency 145
  4.2.7. Preparation of escheriosomes 145-146
  4.2.8. Animals and immunization schedule 146
  4.2.9. Ethics statement 146
  4.2.10. Challenge with Mycobacterial infection and assessment of residual bacterial burden in vital organs 146-147
  4.2.11. Determination of antibody isotype in sera of immunized mice 147
  4.2.12. Isolation of T lymphocytes from spleens of immunized mice 147-148
  4.2.13. Lymphocyte proliferation assay 148
  4.2.14. Cytokine assay: Determination of IFN-γ, IL-4 and IL-12 148-149
  4.2.15. Expression of cell surface markers on immune cells as revealed by FACS analysis 149
  4.2.16. Statistical analysis 149
4.3. Results

4.3.1. SDS-PAGE and Western blot analysis of Rv3620c

4.3.2. Characterization of liposomes

4.3.3. Cytokine assay

4.3.4. IgG2a and IgG1 class switching

4.3.5. Lymphocyte proliferation

4.3.6. Expression of co-stimulatory and memory markers as revealed by flow cytometric analysis

4.3.7. Protective efficacy

4.4. Discussion

5. Bibliography

6. List of Publication