

CONTENTS

INDEX	PAGE NO.
LIST OF FIGURES	i-ii
LIST OF TABLES	iii
LIST OF APPENDIX	iii
LIST OF ABBREVIATIONS	iv-vi
ABSTRACT	vii-ix
REVIEW OF LITERATURE	1-51

CHAPTER 1: ISOLATION OF ANTI-TNF α scFv

1. INTRODUCTION	52-58
2. MATERIAL AND METHODS	59-72
2.1 Rescue of scFv displaying phages and precipitation using PEG/NaCl	
2.2 Titer determination of isolated Phage	
2.3 Panning of scFv library to select hTNF α binders	
2.4 Preparation of scFv-phage for subsequent panning round	
2.5 Preparation of scFv-phage for subsequent panning round	
2.5 Polyclonal and monoclonal phage ELISA	
2.6 High throughput screening and small scale production of soluble Antibody Fragments (scFv antibodies)	
2.7 Selection of Anti-TNF α scFvs based on specificity	

- 2.8 Selection of scFv clones based on avidity
- 2.9 Screening of selected clones by PCR
- 2.10 TNF α neutralization assay
- 2.11 Large scale expression of Recab in *E.coli*
- 2.12 Purification of anti-TNF α scFv (Recab) protein
- 2.13 Stability assay for Recab
- 2.14 Antibody affinity maturation
 - 2.14.1 Construction of randomly mutated yeast scFv display vector
 - 2.14.1 Construction of randomly mutated yeast scFv display vector
 - 2.14. 2 Primary screening of mutated Recab library using fluorescent microplate reader
 - 2.14.3 Microscopy
 - 2.14.4 Flow cytometric analysis of yeast cells

3. RESULTS

73-102

- 3.1 Biopanning of hTNF α specific phage scFv particles
- 3.2 Polyclonal phage ELISA
- 3.3 Screening for specific Binders to hTNF α
- 3.4 Selection of anti-TNF α clones based on Avidity
- 3.5 Characterization of anti-TNF α scFv (Recab) clones
- 3.6 Neutralization of TNF- α -mediated cellular cytotoxicity by Recabs
- 3.7 Large-scale expression of Recab and subsequent purification using

protein A affinity chromatography

3.8 Assay for stability of antigen–antibody association

3.9 Affinity maturation for Recab using Yeast Display System

3.9.1 Construction of Recab surface display yeast strain

3.9.2 Fluorescence microscopy imaging

3.10 Generating large mutant antibody libraries in yeast

3.11 Identification of improved affinity clones

3.12 FACS analysis

3.13 Determination of equilibrium dissociation constant

3.14 Multiple sequence alignment of Recab clones

4. DISCUSSION

103-109

CHAPTER 2 : EXPRESSION OF ANTI-TNF α scFv (REcab) IN YEAST

(Pichia pastoris)

1. INTRODUCTION

110-116

2. MATERIALS AND METHODS

117-127

2.1 Strains and culture conditions

2.2 Transformation of *E. coli* with plasmid DNA

2.3 Isolation of plasmid DNA

2.3.1 Small scale plasmid DNA isolation

- 2.3.2 Medium scale isolation of plasmid DNA
- 2.4 Modifications of plasmid DNA
- 2.5 Transformation of *Pichia pastoris* using LiCl method
- 2.6 Transformation of *P. pastoris* by electroporation
- 2.7 Yeast Genomic DNA Isolation
- 2.8 Southern hybridization analysis
- 2.9 Total RNA extraction and RT-PCR (Reverse Transcriptase Polymerase Chain Reaction)
- 2.10 Generation of Recab expression construct
- 2.11 Screening of Recab transformants of *P. pastoris* using PCR
- 2.12 Expression of Recab in *P. pastoris* transformants
 - 2.12.1 Western blot analysis
- 2.13 Optimization of Recab expression at shake flask level
 - 2.13.1 Effect of methanol concentration on Recab expression
 - 2.13.2 Temperature
 - 2.13.2 Effect of temperature on expression level
- 2.14 Estimation of anti TNF α scFv level using quantitative ELISA
- 2.15 Total proteins estimation (Bradford's method)
- 2.16 Cell based assay

3. RESULTS

128-139

3.1 Expression of Recab in *Pichia pastoris*

3.1.1 Construction of a vector for Recab expression

3.1.2 Confirmation of Recab recombinants of *P. pastoris*

3.1.3 Analysis of Recab expression in *P. pastoris*

3.2 Optimization of Recab expression

3.2 Analysing effect of Temperature and Methanol concentration on Recab expression

3.3 Cell based assay for Recab protein obtained from *P. pastoris* transformants

4. DISCUSSION	140-143
5. SUMMARY	144-147
6. APPENDIX	148-154
PATENT	155-159
BIBLIOGRAPHY	160-186