CHAPTER 5

BIOCHEMISTRY

Doctoral Theses

029. CHARANPREET KAUR
Development of Efficient Recombinant Antigens and Antibodies for Improved Immuno-Diagnosis of Tuberculosis.
Supervisor : Prof. Vijay K. Chaudhary
Th 22332

Abstract

Time-tested immunochemical tests to detect pathogen-specific antigen or antibodies in body fluids have not been successful for Tuberculosis (TB). Antigen detection could be a potential alternative to conventional microbial confirmation. The work embodied in this thesis reports the production of recombinant reagents for detection of two M. tuberculosis specific secreted proteins, MPT64 (Rv1980c) and MPT63 (Rv1926c). The DNA encoding MPT64 and MPT63 were cloned, and proteins expressed and purified as decahistidine-tagged molecules. Hybridomas secreting anti-MPT64 and anti-MPT63 monoclonal antibody (MAbs) were produced. Sandwich ELISA and pairwise mapping by surface plasmon resonance (SPR) delineated non-competing MAb pairs. Sandwich ELISAs were developed detecting MPT64 and MPT63 with detection limit of 37 and 14 pg/ml respectively. The DNA encoding variable domains of MAbs was PCR-amplified employing cDNA from hybridoma as template. Strategies were developed for cloning functional V_L and V_H as phage-displayed scFv, despite presence of abundant aberrant kappa chain. In vivo and in vitro targeted enzymatic biotinylation systems were developed employing E. coli Biotin Protein ligase (BirA). In vivo biotinylation system was optimized by co-expressing soluble protein carrying BAP-tag and BirA under regulated promoter. For in vitro biotinylation, deca-histidine-tagged and tag-less BirA were cloned, expressed and purified. Using sequences of V_L and V_H, Light chain (LC) and Fd-BAP (BAP-tag, GLNDIFEAQKIEWHE) were assembled and expressed in E. coli as inclusion bodies, which were employed for obtaining recombinant soluble Fab molecules with C-terminal BAP tag. The purified Fab-BAP was biotinylated in vitro and used as capture molecules upon immobilization on streptavidin (SA)-coated plates with detection using HRP-conjugated MAbs. Thus, in conclusion, the thesis describes a complete technology to obtain functional variable domains of MAbs from hybridoma even in presence of aberrant light chain and to produce recombinant Fab molecules with site-specific biotinylation.

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030. GROVER (Payal)

Recombinant Mycobacterial Proteins and Antibodies for Immunochemical Detection of Mycobacteria in Culture.

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Abstract

Unraveling the pathogen-specific antigen could be a realistic alternate for demonstrating the presence of the disease-causing pathogen. In this context, presence of mycobacterial specific-antigen(s) in the growing culture could be considered as a possible diagnostic test to confirm the presence of M. tuberculosis [belonging to Mycobacterium tuberculosis complex (MTC)] and non-tuberculous mycobacteria (NTM). The present work emphasizes on development of immunochemical reagents for detecting clinically relevant mycobacteria by production and characterization of monoclonal antibodies (MAbs) against Ag85A and Ag85B of M. tuberculosis and Ag85A of NTMs. Ag85A (Rv3804c), Ag85B (Rv1886c) and MPT51 (Rv3803c) of M. tuberculosis were purified as decahistidine-tagged monomeric proteins and employed for producing MAbs. Highly sensitive sandwich ELISAs were developed for simultaneous and individual detection of Ag85A and Ag85B with analytical detection limit of 37-111 pg/ml. Additionally, tag-less Ag85A and Ag85B of M. tuberculosis, and Ag85A homologs of NTMs were produced in E. coli and employed for producing antibodies, which could bind to Ag85A of NTMs and M. tuberculosis in species-specific or NTM group-specific manner. This study identified combinations of MAbs for developing ELISA for detection of; a) both MTC and NTMs [(i) combination of MAbs BC03-1 and 85-12 as capture and detection using biotinylated MAb 85A03 detected Ag85A and Ag85B of M. tuberculosis and Ag85A of all NTMs tested except M. fortuitum, (ii) capture on MAb BC01-2 with detection using biotinylated MAb BC17-1 detected Ag85A from M. fortuitum] b) mycobacteria belonging to MTC (capture using MAbs 85-14 or 85-11 and detection using biotinylated MAb 85-12 resulted in specific detection of Ag85A and Ag85B of M. tuberculosis, respectively with no cross-reactivity with Ag85A from four NTMs tested). The reagents developed in this work should find use in developing rapid tests for confirming presence of all clinically relevant species of Mycobacteria, and differentiate between NTM and MTC.

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