شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

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قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
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Use of Some Mycobacterial Peptides and Recombinant Proteins for Diagnosis of Bovine Tuberculosis

A thesis Submitted by

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Abstract

In this study, some ***M. bovis*** recombinant peptides "recombinant early secretory antigenic target-6 (rESAT-6), recombinant major protein of ***M. bovis*** 64 (rMPB-64) and recombinant major protein of ***M. bovis*** 83 (rMPB-83)" were cloned, expressed and purified from well identified of ***M. bovis*** strains. They were used in both serum dependent tests as ELISA and whole blood techniques; IFN- γ assay as a diagnostic tests for tuberculosis. So, we successfully cloned and expressed the rESAT-6, rMPB-64 and rMPB-83 proteins in pure form and identified by SDS-PAGE and immunoblotting technique. They were used in skin testing in infected guinea pigs by killed ***M. bovis*** and it showed clear response using different used doses of expressed proteins as well as it was used in ELISA on 500 serum samples from tuberculin tested animals and in Gamma interferon assay on 50 tuberculin tested animals in comparison with PPD. The obtained results proved the antigenicity and immunogenicity of the recombinant antigens as they can detect cases as tuberculous positive from tuberculin negative cases by ELISA and gamma interferon assay. The sensitivity and specificity of each used test were recorded and discussed. Results showed higher sensitivity and specificity of purified recombinant proteins over PPD when used in diagnosis of bovine tuberculosis in ELISA and IFN-γ assay and superiority of these tests over tuberculin test. It was concluded that the purified rESAT-6, rMPB-64 and rMPB-83 were implementable as a specific and sensitive antigens alternative to ordinary diagnostic PPD. It also declare that use of this antigen resulted in a marked improvement in the ELISA and IFN-γ release among both TST negative and TST positive cattle.

Keywords: ***M. bovis***, ELISA, Gamma interferon, ESAT-6, MPB-83, MPB-64, bovine tuberculosis.

Dedication

To
My Father's Spirit, My dear Mother,

My Brothers, My Sisters, their children,

My Wife

And

My Friends

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Wagdy Samir Beshir

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List of Abbreviations

AFB	Acid Fast Bacilli
Ag85	Antigen 85
BCG	Bacillus Calmette Guerine
BTB	Bovine Tuberculosis
CFP-10	Culture Filtrate Protein 10
CFU	Colony forming unit
CITT	Comparative Intradermal Tuberculin Test
CMI	Cell Mediated Immunity
ELISA	Enzyme linked immunosorbent assay
ESAT-6	Early Secretory Antigenic Target 6
EU	European Union
HIV	Human immune deficiency virus
IFN-γ	Interferon Gamma
IgG	Immunoglobulin G
IGRA	Interferon gamma release assay
IT	Intradermal tuberculin
kDa	Kilo Dalton
L.N.	Lymph node
LAM	Lipoarabinomannan
L-J	Lowenstein-Jensen medium
MOTT	Mycobacteria Other Than Tuberculosis
MPB-64	Major Protein of ***M. bovis*** 64
MPB-83	Major Protein of ***M. bovis*** 83
MoAbs	Monoclonal antibodies
MTC	*Mycobacterium Tuberculosis* Complex
MW	Molecular weight
Mtb	***Mycobacterium tuberculosis***
NK	Natural Killer Cells
NTM	Non-Tuberculous Mycobacteria
NVL	Non-Visible Lesions
OADC	Oleic Albumin Dextrose Catalase
OIE	Office International Des Epizooties
PCR	Polymerase chain reaction
PPD	Purified Protein Derivative
PPD-A	Avian Purified Protein Derivative

PPD-B	Bovine Purified Protein Derivative
RD	Region of difference
RT	Real Time
SCT	Single Cervical Tuberculin Test
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SICTT	Single Intradermal Comparative Tuberculin skin Test
ST-CF	Short Term Culture Filtrate
TB	Tubercle Bacilli
TST	Tuberculin Skin Test
UK	United Kingdom
WHO	World Health Organization
ZN	Ziehl-Nelseen