Mycobacterial mycolic acids as immunoregulatory lipid antigens in the resistance to tuberculosis

By Dismore Gilbert Ramathudi Siko

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"Morena ke modisa wa ka ga nkitla ke tlhoka sepe, o ntsamaisa mo mafulong a matalana"

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List of Abreviations

β2M	Beta-2-microglobulin
γδ	Gamma-delta
A	
AIDS	Acquired immune deficiency syndrome
AM	Alveolar macrophage
APC	Antigen presenting cell
ATCC	American Type Culture Collection
B	
BCG	Bacillus Calmette-Guérin
C	
CAM	Cytokine-activated monocytes
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CFU	Colony forming units
Chol	Cholesterol
CMI	Cell-mediated immunity
D	
DEPC	Diethyl pyrocarbonate
DN	Double negative
DNA	Deoxyribonueleic acid
DOTS	Directly observed treatment short course

DTH	Delayed-type hypersensitivity
E	
E. coli	*Eschirichia coli*
EDTA	Ethylenediaminetetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EMB	Ethambutol
G	
GAP-DH	Glyceraldehyde-3-phosphate dehydrogenase
GLC	Gas-liquid chromatography
GLP	Good laboratory practise
GM-CSF	Granulocyte macrophage colony stimulating factor
H	
HCl	Hydrochloric acid
HEPA	High Efficiency Particulate Air
HIV	human immunodeficiency virus
HPLC	High-performance liquid chromatography
I	
i.n.	Intranasal
i.p.	Intraperetoneal
i.v.	Intravenous
IFN-γ	Interferon gamma
IgG	immunoglobulin G
IL-	Interleukin

INH	Isiniazid
iNOS	Inducible nitric oxide synthase
IRMA	Immunoradiometric assay
K	
KOH	Potassium hydrooxide
L	
LAM	lipoarabinomannan
LDL	Low-density lipoproteins
LPS	lipopolysaccharides
M	
M. avium	*Mycobacterium avium*
M. bovis	*Mycobacterium bovis*
M. leprae	*Mycobacterium. leprae*
M. tuberculosis	*Mycobacterium tuberculosis*
M.tb	*Mycobacterium tuberculosis*
MA	Mycolic acids
MAIDS	Murine acquired immunodeficiency syndrome
MCF	Mink cell focus forming
MDR TB	Multi-drug resistant tuberculosis
$MgCl_2$	Magnesium chloride
MHC	Major histocompatibility complex
MOPS	3-(*N*-morpholino) propanesulfonic acid
MOTTS	Mycobacteria Other Than Tuberculosis

MRC	Medical Research Council
mRNA	Messenger RNA
MuLV	Murine leukemia virus
MΦ	Macrophage
N	
NAC	No amplification control
NaCl	Sodium chloride
NALC-NaOH	N-acetyl-L-cysteine-sodium hydroxide
NaOH	Sodium hydroxide
NK	Natural killer cells
NKSF	Natural killer cell stimulatory factor
NO	Nitric oxide
NTC	No template control
O	
OD	Optical density
P	
PBS/AE	Phosphate buffered saline azide EDTA
PC	Phospahatidyl choline
PCR	Polymerase chain reaction
PMBC	Peripheral blood mononuclear cells
PPD	Purified protein derivative
PZA	Pyrazinamide

R

RFP	Rifampetine
RIA	Radioimmunoassay
RIF	Rifampicin
RNA	Ribonucleic acids
RNI	Reactive nitrogen intermediates
ROI	Reactive oxygen intermediates
RQ-PCR	Real time quantitative PCR
RQ-RT-PCR	Real-time quantitative reverse transcriptase PCR
rRNA	Ribosomal ribonucleic acids
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase PCR
RZL	Rifalazil

S

Sal	Saline
SC-1	Stromal cell line
Ser	Serum
SIV	Simian immunodeficiency virus
SM	Streptomycin
SOD	Superoxide dismutase
SQ-RT-PCR	Semi-Quantitative RT-PCR

SR	Scavenger receptor
T	
TB	Tuberculosis
TCR	T cell receptor
Th	T helper
TNF-α	Tumor necrosis factor alpha
W	
WHO	World Health Oraganisation

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Summary: Mycobacterial mycolic acids as immunoregulatory lipid antigens in the resistance to tuberculosis

By: Dismore Gilbert Ramathudi Siko
Promoter: Prof. Dr. J.A. Verschoor
Co-promoter: Dr E. Johannsen
Department: Biochemistry
Qualification: Doctor Philosophiae (Biochemistry)

Tuberculosis has returned with vengeance mainly due to the resurgence of multi drug resistant strains incurred by non-compliance to the 6-9 months chemotherapy programme. Co-infection with HIV, which disorientates the immune response, has aggravated the situation. This study was built on previous observations that indicated that the major lipid cell wall component of *M. tuberculosis*, i.e. mycolic acids, a wax that envelopes and protects the bacillus from the hostile host immune system, can be purified and administered to animals for protection against subsequent tuberculosis induction. It was established in this study that mycolic acids pre-treatment can significantly protect mice upon subsequent intranasal infection with *M. tuberculosis* and that this protection is not attributed so much to the T helper cell immunity, but rather through induction of innate immunity. In the murine AIDS model, innate immunity induced by mycolic acids pre-treatment was not enough to protect the virally immunocompromised mice against subsequent *M. tuberculosis* infection. Mycolic acids administration in mice did not support tuberculosis chemotherapy to enable shortening of the duration of chemotherapy. In human tuberculosis patients, antibodies to mycolic acids could be measured in a specially adapted configuration of a resonant mirror biosensor. The preliminary investigation opened up the possibility that the prevalence of anti-mycolic acids

antibodies in tuberculosis patients may be measured as a surrogate marker for tuberculosis infection. An apparent cross-reactivity between mycolic acids and cholesterol in binding to tuberculosis patient antibodies may provide far reaching insight in the role of the mycolic acids in the cell wall to facilitate infection. This research contributed significantly to the understanding of the host-pathogen interaction in tuberculosis, to open up fresh approaches to improved diagnosis and chemotherapy.

Opsomming: Mikobakteiële mikolsure as immunregulatariese lipied-antigene in die weerstandigheid teen tuberkulose.

Deur: Dismore Gilbert Ramathudi Siko
Promotor: Prof. Dr. J.A. Verschoor
Medepromotor: Dr. E. Johannsen
Departement: Biochemie
Kwalifikasie: Doctor Philosophiae (Biochemie)

Tuberkulose is terug met mening, hoofsaaklik vanweë die herlewing van multi-drogery-weerstandige patogeenstamme deur die onvoldoening aan die 6-9 maande lange chemoterapie-termyn. Ko-infeksie met HIV, wat die immuunrespons versteur, het die toestand vererger. Hierdie studie is gebaseer op vroeëre waarnemings wat daarop gedui het dat die dominante lipied-selwandkomponent van *M. tuberculosis*, d.i. mikolsure - 'n was wat die basillus omhul en beskerm teen die vernietigende immunstelsel van die gasheer - gesuiwer en toegedien kan word aan proefdiere om beskerming te verleen teen daaropvolgende tuberkulose-induksie. Daar is met hierdie studie vasgestel dat voor-infeksie behandeling met mikolsure muise aansienlik kan beskerm teen daaropvolgende intranasale infeksie met *M. tuberculosis* en dat hierdie beskerming nie soseer te wyte is aan die T-helpersel-immuniteit nie, maar eerder deur die induksie van ingeskape immuniteit. In die muis-VIGS-model, was die ingeskape immuniteit, geïnduseer deur voorbehandeling met mikolsuur, onvoldoende om teen *M. tuberculosis* infeksie te beskerm in die virale immuunverydelde muis. Toediening van mikolsure aan muise het nie die effektiwiteit van chemoterapie ondersteun om 'n verkorte duur daarvan moontlik te maak nie. In mens tuberkulose-pasiënte kon teenliggame teen mikolsure aangetoon word in 'n spesiaal aangepaste konfigurasie van die resonante spieëlbiosensor. Die voorlopige ondersoek het die moontlikheid onthul dat die bestaan van anti-mikolsuur-teenliggame in tuberkulose pasiënte gemeet kan word as 'n surrogaatmerker van tuberkulose infeksie. 'n Oënskynlike kruis-reaktiwiteit tussen mikolsure- en cholesterol-binding aan tuberkulose-pasiënt-teenliggame kan verreikende insig verleen aan die rol

van mikolsure in die selwand om infeksie te bevorder. Hierdie navorsing het bygedra tot 'n beter begrip van die gasheer-patogeen interaksie in tuberkulose, ten einde vars benaderings te skep vir verbeterde diagnose en chemoterapie.