

**Immunological properties of mycolic
acids, the major lipid cell wall
component of *Mycobacterium
tuberculosis***

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Summary

The immunological effects of mycolic acids (MA) from *Mycobacterium tuberculosis* on mouse peritoneal macrophages were studied. MA was solubilized using various carriers. Phagosome uptake and maturation (into late stage phagolysosomes) were compared using fluorescent markers and the confocal microscope. During assessment on the effects of MA on mouse macrophages, changes in morphology and activation of the macrophages were found. This indicated that the MA was immune reactive towards macrophages. The phenotype of cell that develops after *in vivo* loading with MA was characterized by using cell surface markers: it was found that MA-loaded macrophages developed into foam cells. Cell survival, proliferation and macrophage cytokine production were examined to characterize the foam-like cells. The effect of MA-induced foam-like cells on living *Mycobacterium tuberculosis* was evaluated and increased bactericidal activity was found. The roles of reactive oxygen and nitrogen intermediates via myeloperoxidase were also examined and a theoretical mechanism for the formation of foam cells proposed. The possible role of myeloperoxidase in activation of macrophages, foam cell formation and killing of *Mycobacterium tuberculosis* is discussed. It is postulated that a possible relationship might exist between tuberculosis and atherosclerosis that is facilitated by mycolic acids.

Opsomming

Die immunologiese effekte van mikoosuur (MA) geïsoleer vanaf *Mycobacterium tuberculosis* op muis peritoneale makrofaag selle is ondersoek. Mikoosuur is in oplossing gebring deur van verskeie draers gebuikte maak. Fagositose en maturasie van die fago-lisosoom is met behulp van fluoresente merkers en die konfokale mikroskoop ondersoek. Ondersoek na die effek van mikoosuur op makrofae, het aan die lig gebring dat makrofae verandering in aktivering en morfologie ondergaan. Dit het die immunologiese aktiwiteit van mikoosuur op makrofae aangetoon. Die fenotipe van die selle wat deur mikoosuur geïnduseer is, is met behulp van oppervlakte merkers ondersoek: daar is bevind dat mikoosuur-gelaaide makrofae in skuimselle ontwikkel. Selproliferasie, -oorlewing en sitokienproduksie is ook ondersoek ten einde die skuimselle te karakteriseer. Die effekte van MA-geïnduseerde skuimselle op lewende *Mycobacterium tuberkulose* is getoets en 'n verhoogde bakteriosidiese effek is gevind. Die moontlike rol van miëloperoksidase in die aktivering van makrofae, skuimselvorming en uitwissing van *Mycobacterium tuberkulose* word bespreek. Die rol van reaktiewe suurstof en stikstof radikale op lewendige tuberkulose basille is ook ondersoek en 'n teoretiese meganisme vir die ontstaan van die skuimselle is gepostuleer. Soos reeds bewys is vir ander organismes, is die vraag of tuberkulose ook moontlik 'n rol mag speel in aterosklerose.

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Abbreviations

A:

ACAT	: acyl co-enzyme A cholesterol-acyltransferase
ACEH	: acid cholesterol ester hydrolase
Ag	: antigen
AhpC	: alkyl hydroperoxidase reductase protein
AIDS	: acquired immunodeficiency syndrome
APC	: antigen presenting cell
ARDS	: adult respiratory distress syndrome
ATP	: adenosine triphosphate
Av-HRP	: Avidin-Horseradish peroxidase
AZT	: 3'-azido 3'-deoxythymidine

B:

BCG	: Bacille Calmette Guerin
BMF	: (5 -bromo) methylfluorescein
BSA	: bovine serum albumin

C:

CD	: cyclodextrin
CE	: cholesterol ester
CEH	: cholesterol ester hydrolase
CH	: carbohydrate
CLPS	: <i>Chlamydia pneumonia</i> lipopolysaccharide
CMC	: critical micellar concentration
Cp	: ceruloplasmin
CR	: complement receptor
CTL's	: cytotoxic T cells

D:

DLIPC	: dilinoleoyl phosphatidylcholine
DLPC	: dilauroyl phosphatidylcholine
DMF	: dimethylformamide
DN	: double negative



DNA	: deoxyribonucleic acid
DNP-Cap-PE	: dinitrophenyl-epsilon-aminocaproyl phosphatidyl
DSPC	: distearyl phosphatidylcholine
DTH	: delayed-type hypersensitivity
E:	
EE	: early endosome
ELISA	: enzyme linked immunosorbent assay
ER	: endoplasmic reticulum
EtOH	: ethanol
F:	
FACS	: fluorescence activated cell sorter
FasL	: Fas-ligand
FAT	: fatty acid translocase
FBS	: foetal bovine serum
FC	: free cholesterol
FCS	: foetal calf serum
FITC	: fluorescein-iso-thiocyanate
G:	
α GalCer	: α galactosylceramide
GM-CSF	: granulocyte monocyte colonizing factor
GMM	: glucose monomycolate
H:	
HIV	: human immunodeficiency virus
HOX	: hypohalous acid
HPLC	: high performance liquid chromatography
HS	: hydroxystearate
I:	
LDL	: intermediary low density lipoprotein
IL	: interleukin
IFN γ	: interferon γ
IP	: intraperitoneal

ISPF	: isonitrosopropiophenone
IV	: intravenous
K:	
katG	: catalase-peroxidase protein
L:	
LAM	: lipoarabinomannan
LAMP	: lysosome associated membrane protein
LDL	: low density lipoprotein
LE	: late endosome
LipoMA	: liposome-mycolic acid
LO	: lipo-oxygenase
LPL	: lipoprotein lipase
LPS	: lipopolysaccharide
LRP	: LDL receptor-related protein
Lyso-PC	: lysophosphatidylcholine
m/v	: mass/volume
M:	
MA	: mycolic acid
mAU	: milli-absorption units
MAC	: maximum additive concentration
MBP	: mannose-binding protein
M-CSF	: macrophage colony stimulating factor
MDR	: multi drug resistance
MHC	: major histocompatibility complex
MIIC	: MHC class II compartment
MP	: mononuclear phagocytes
MPO	: myeloperoxidase
MR	: mannose receptor
MTP	: microsomal transfer protein
MW	: molecular weight

N:

- NADP : nicotinamide adenine dinucleotide
NBCS : newborn calf serum
NCEH : neutral cholesterol ester hydrolase
NK-cells : natural killer cells
NO : nitric oxide
NOS : nitric oxide synthetase
NRAMP : natural-resistance-associated macrophage protein

O:

- OD : optical density
oxLDL : oxidized low density lipoprotein

P:

- PBM : peripheral blood monocyte
PBS : phosphate buffered saline
PC : phosphatidylcholine
PCR : polymerase chain reaction
PCv/v : packed cell volume/volume
PEC : peritoneal exudate cell
PEG : polyethylene glycol
PGE₂ : prostaglandin E₂
PI : propidium iodide
PI₃ : phosphatidylinositol 3
PLA₂ : phospholipase A₂
PLGA : poly lactic-coglycolic acid
PPD : purified protein derivative

R:

- RT-PCR : reverse transcriptase polymerase chain reaction
RES : reticulo-endothelial system
RNI : reactive nitrogen intermediates
ROI : reactive oxygen intermediate
RT : room temperature

S:

sCD14 : soluble CD14
SDS : sodium dodecyl sulphate
SMase : sphingomyelase
SP-A : surfactant protein A
SP-D : surfactant protein D
SR : scavenger receptor

T:

TAP : transporter associated with antigen presentation
TB : tuberculosis infection
TCR : T cell receptor
TdR : [methyl-³H] thymidine
TG : triglyceride
TGFβ : transforming growth factor β
TGN : trans-golgi network
Th1/2 : T-helper 1 or 2 cell
TI : thymus independent
TNF : tumour necrosis factor

U:

UK : United Kingdom
USA : United States of America
v/v : volume/volume

V:

VLDL : very low density lipoprotein

W:

WHO : World Health Organization