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MASSEY UNIVERSITY

APPLICATION FOR APPROVAL OF REQUEST TO EMBARGO A THESIS

(Pursuant to AC 98/168 (Revised 2), Approved by Academic Board 16.02.99)

Name of Candidate: Carol Jobsis ID Number: 97087628

Degree: M.Sc. Dept/Institute/School: IMBS

Thesis Title: THE IMMUNOMODULATORY ROLE OF LIPOTEICHOIC ACID IN PROBIOTIC BACTERIA

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As author of the above named thesis, I request that my thesis be embargoed from public access

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The Immunomodulatory Role of Lipoteichoic Acid from Probiotic Bacteria

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science
in
Biochemistry

Institute of Molecular BioSciences
Massey University
Palmerston North, New Zealand.

Carel Michael Hutchings Jöbsis

2011

Abstract

Different probiotics provide different health benefits, and some of these may be explained by immunomodulatory activity. These immunomodulatory effects can vary between different probiotic strains and microbial-associated molecular patterns (MAMPs) may be responsible for this variation. One MAMP, lipoteichoic acid (LTA), is a macroamphiphile associated with the cell surface of gram positive bacteria. LTAs from different strains of bacteria have been shown to induce different immunomodulatory profiles.

LTA was purified from three strains of lactic acid bacteria (LAB) that are known to elicit different immune responses, then analysed for immunomodulatory activity using human cell based assays. The activity of each LTA was shown to reflect elements of the immunomodulatory profile of the original strain. The structure of each LTA was determined using NMR (nuclear magnetic resonance spectroscopy). Structural differences found between the LTAs were compared to the differences in their immunomodulatory behaviours, showing that the differing structures may be responsible for strain-specific immune profiles. It has been previously shown that inactivation of the *dlt*D gene in an established probiotic strain of LAB results in changes to the immune effects induced by the mutant bacterial cell compared to the wild type. This study has shown using NMR analysis that the structure of LTA isolated from this mutant strain is altered, reflecting the distinct immune profile of the mutant bacteria.

LTAs from the three strains in this study were found to contain N-acetyl-glucosamine substituents, which have previously been found only on highly pro-inflammatory LTAs, e.g., those from *Staphylococcus aureus*. LTAs from the three strains were also shown to contain unsaturated fatty acids, which have so far been found in the LTAs of only LAB, including three other probiotic strains. These structural features may explain some of the immunomodulatory effects observed for these strains. It was found that isolated LTA may not be as effective at inducing immune responses as LTA on cells. Further exploration of potential interactions of LTA with other MAMPs, and other factors that may alter the presentation of LTA to immune cells in the case of intact cells is necessary to fully understand the role of LTA in immunomodulation.

For Carel Otto Jöbsis
1925 - 2011

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Abbreviations

In addition to *le Système international d'unités* (SI) and the derived SI units, the chemical symbols from the Periodic Table of Elements, the standard amino acid abbreviations and the cytokine abbreviations as given by Delves *et al.* (2011), the following abbreviations were used :

~	Approximately
1D	One dimensional
2D	Two dimensional
A, Abs	Absorbance
AcO^-	Acetate ion
amt	Amount
APC	Antigen presenting cell
CBA	Cytometric bead array
CD4	Cluster of Differentiation 4
CD14	Cluster of Differentiation 14
CD36	Cluster of Differentiation 36
CFA	Cyclopropane fatty acid
cfu	Colony forming units
CMC	Critical micelle concentration
concn	Concentration
COSY	Correlation Spectroscopy
CV	Column volumes
D_2O	Deuterium oxide (heavy water)
DAG	Diacylglycerol
D-ala	D-alanine
DC	Dendritic cell
DNA	2'-deoxyribonucleic acid
*dlt*A to E	The operon responsible for D-alanylation of LTA
DQF-COSY	Double Quantum Filtered Correlation Spectroscopy
ELISA	Enzyme-linked immunosorbent assay
EPS	Exopolysaccharide
ESI FT-MS	Electro-spray ionisation Fourier transform-mass spectrometry
EU	Endotoxin units
FA	Fatty acid
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal bovine serum

FID	Free Induction Decay
FPLC	Fast Protein Liquid Chromatography
g	Gravity
Gal	Galactose
GBS	Group B *Streptococcus*
GC-MS	Gas Chromatography –Mass Spectrometry
GlcNAc, GNAc	N-acetyl-glucosamine
Gro	Glycerol
GroP	Glycerol-phosphate
HIC	Hydrophobic Interaction Chromatography
HOD	Hydrogen-Oxygen-Deuterium (semi-heavy water)
HPAEC-PAD	High-performance anion-exchange chromatography with pulsed amperometric detection
HSQC	Heteronuclear Single Quantum Correlation
IBD	Inflammatory bowel disease
IFN-γ	Interferon gamma
IL	Interleukin
LAB	Lactic acid bacteria
LAL	Limulus Amoebocyte Lysate
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MAMP	Microbe-associated molecular pattern
MDP	Muramyl dipeptide
MQ	Milli-Q highly purified water
MS	Mass Spectrometry
N	Normal (for an acid, the concn of H+ ions in mol/L)
n.d.	Not determined
NK	Natural Killer lymphocyte
NMR	Nuclear magnetic resonance spectroscopy
NOD	Nuclear oligeromisation domain
NOESY	Nuclear Overhauser effect spectroscopy
OD	Optical density
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PGN	Peptidoglycan
PGP	Poly(glycerol-phosphate)
pH	Activity of the H^+ ion, where pH = -log[H^+]
PNH	Phosphate non-hydrolysed (measured with rapid method)
ppm	Parts per million

PRR	Pattern recognition receptor
PtdG	Phosphatidyl-glycerol
rDNA	Ribosomal DNA
RFU	Relative fluorescence units
rpm	Revolutions per minute
RPMI	A media for human cell culture (named for Roswell Park Memorial Institute)
SEM	Standard error of the mean
SFA	Saturated fatty acid
S/N	Signal to noise ratio
TA	Teichoic acid (encompassing LTA and WTA)
Th cell	Helper CD4+ T lymphocyte
TNF	Tumour Necrosis Factor
TLR	Toll-like receptor
TOCSY	Total Correlation Spectroscopy
Treg	Regulatory CD4+ T lymphocyte
UFA	Unsaturated Fatty Acid
UV	Ultraviolet (light)
WT	Wild type
WTA	Wall teichoic acid
w/v	Weight per volume
v/v	Volume per volume