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Further insight into the Lewis histo-blood-group system as
revealed from study of Polynesian and Caucasian plasma
and erythrocyte glycosphingolipids

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A thesis submitted in partial fulfilment
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This thesis is dedicated to
my wife Helen
and my children Jessie and Zak

Abstract

This project involved the study of Lewis and related blood group glycosphingolipid isolated from individuals with normal and aberrant Lewis/secretor phenotypes. The objective was to find a biochemical basis for the unusual expression of Lewis and secretor phenotypes in Polynesians and to use this information to shed light on the "normal" expression of Lewis antigens.

By using purified glycolipids, presenting them in the cell free environment of thin layer chromatography to Lewis antibodies and by determining structures by mass spectrometry it has been shown that:

1. The Le^c epitope is a terminal Gal β 1-3Gal sequence, and not an internal branch as proposed by Hanfland (Hanfland *et al.*, 1986).
2. Le^c or H-5-1 are present in Lewis negative phenotypes and their consequent consumption by the *Le* and *Se* transferases resulting in the known Le^a and Le^b antigens can be seen in the Lewis positives.
3. Phenotypically Le(a-b-) individuals have small amounts of Lewis antigens. This clearly demonstrates that although the Lewis negative phenotype exists at the crude serological level, this phenotype is not an "all-or-nothing" phenomenon at the chemical level. This also allows it to be postulated that the *le* gene is probably partially active.
4. Le(a+b+) individuals have both Le^a and Le^b glycolipids in the erythrocyte membrane and in plasma. Observed phenotyping anomalies appear to be related to there being quantitatively less Le^b-6 in the Polynesian Le(a+b+) erythrocyte membrane than in the Le(a-b+) membrane.
5. The Le(a+b-) phenotype of Polynesians is actually the Le(a+b+) phenotype but with serologically undetectable Le^b. This allows it to be postulated that the nonsecretor gene (*se*) is absent in Polynesians.
6. Extended structures are present in most of the Polynesian samples which is in support of a postulated weak secretor gene (*Se^w*). It now appears that the difference between the extended Lewis glycolipids of Caucasians and Polynesians is quantitative. The postulated *Se^w* transferase appears to be inefficient and allows for increased formation of elongated glycoconjugates (polyglycosylceramides) to result.
7. Reduced fucosyltransferase activity allows increased elongation of the precursor chain to occur, which allows it to be postulated that fucosylation of the precursor prevents, or at least markedly reduces, chain elongation. It is speculated that, as almost everyone is either Lewis and/or secretor positive, perhaps the prevention of chain elongation is a biological reason as to why the Lewis and Secretor polymorphisms exist.
8. Differences in ceramide patterns of Lewis active glycolipids suggests that the small intestinal tract is not the only origin of plasma glycolipids, or there is differential absorption.
9. There is no plasma glycolipid-based reason for there being increased H type 2 antigen reactivity in the Polynesian erythrocyte membrane, nor a reason for the H antigen association with the Le(a+b+) phenotype.

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Abbreviations

amu	atomic mass unit
BSA	bovine serum albumin
C/M/H	solvent mixes of chloroform/methanol/water
CBA	chromatogram binding assay
Cer	ceramide
dHex	deoxyhexose (fucose)
EI	electron impact
FA	fatty acid
FAB	fast atom bombardment
Fuc	fucose
Gal	galactose
GalNAc	<i>N</i> -acetylgalactosamine
Glc	glucose
GlcNAc	<i>N</i> -acetylglucosamine
h	hydroxy (with reference to ceramide fatty acid residues)
Hex	hexose
HexNAc	<i>N</i> -acetylhexosamine
HPLC	high pressure liquid chromatography
HPTLC	high-performance thin-layer chromatography
Imm	immonium
LCB	long chain base
M	molecular
<i>m/z</i>	mass to charge
MAB	monoclonal antibody
MS	mass spectrometer or spectrometry
n	normal (with reference to ceramide fatty acid residues)
NeuAc	neuraminic acid, or sialic acid
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
ppm	parts per million
TLC	thin layer chromatography (high performance)

Commercial names

Kimax	Kimble, Illinois, USA
Mallinckrodt	Mallinckrodt Specialty Chemicals Co., Kentucky, USA
Merck	Merck, Darmstadt, West Germany
Riedel-de Haën	Riedel-de Haën AG, Seelze, West Germany
Schleicher and Schuell	Schleicher and Schuell, Dassel, West Germany
Spectrapor	Spectrum Medical Industries Inc, Cal, USA
Visking	Medicell International Ltd, London
Whatmans	Whatmans Laboratory Division, Maidstone, England