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Expression of Lewis histo-blood group glycolipids in the plasma of individuals of Le(a+b+) and partial secretor phenotypes

STEPHEN M. HENRY[1*,2], RAFAEL ORIOL[3] and BO E. SAMUELSSON[1]

[1]*Department of Clinical Chemistry and Transfusion Medicine, University of Göteborg, 41345, Sweden*
[2]*Department of Transfusion Medicine, Auckland Regional Blood Centre, Auckland, New Zealand*
[3]*INSERM U178, Villejuif 94807 Cedex, France*

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Red cell Lewis antigens are carried by glycosphingolipids passively absorbed from plasma. Plasma was collected from a spectrum of individuals with normal and unusual Lewis/secretor phenotypes in order to investigate the glycolipid basis for the unusual phenotypes. Samples were obtained from: a Le(a+b−) ABH nonsecretor who secreted Lewis substances; a Le(a+b−) partial secretor; Le(a+b+) partial secretors; Le(a+b+) secretors; and a full range of normal Lewis/secretor phenotypes as controls. The Le(a+b+) samples represented Polynesian, Asian and Réunion Island ethnic backgrounds. Nonacid glycolipids were prepared, separated by thin-layer chromatography, and then immunostained with potent monoclonal antibodies of known specificity. Despite different serological profiles of the Le(a+b−) and Le(a+b+) Polynesian samples, their plasma glycolipid expressions were very similar, with both Le^a and Le^b co-expressed. The copresence of Le^a and Le^b in Le(a+b+) samples is in marked contrast to Caucasians with normal Lewis phenotypes, who have predominantly either Le^a or Le^b. These results suggest that there is a range of the secretor transferases in different individuals, possibly due to different penetrance or to several weak variants. We also show that Lewis epitopes on longer and/or more complex core chains appear to be predominant in the Polynesian Le(a+b+) samples. The formation of these extended glycolipids is compatible with the concept that in the presence of reduced secretor fucosyltransferase activity, increased elongation of the precursor chain occurs, which supports the postulate that fucosylation of the precursor prevents or at least markedly reduces chain elongation.

Keywords: Lewis antigens, glycolipids, Le(a+b+) plasma, secretor

Abbreviations: CBA, chromatogram binding assay; TLC, thin-layer chromatography.

Introduction

The Lewis system is structurally related to the ABH blood group system and belongs to a single family of oligosaccharides with related chemical structures (reviewed in [1]). Like ABH, Lewis and related antigens are present in all organs of the human body and the term histo-blood group antigens is now used to describe these antigens [2].

The Lewis system at the red cell phenotypic level is comprised of two major antigens, Le^a and Le^b, which were respectively first described by Mourant and Andresen [3, 4]. It is believed that exocrine epithelial cells, mostly of endodermal origin, synthesize the Lewis antigens and these antigens are shed into exocrine secretions and plasma [reviewed in 1]. The plasma glycolipids are then acquired by cells of the peripheral circulation [5–7]. The ability to express Lewis antigens, Le^a and/or Le^b, is dependent on an individual's Lewis genotype, while the type and amount of these Lewis antigens expressed is dependent on the individual's secretor genotype [8, 9]. The term 'secretor' is used to describe those individuals in whose saliva can be found large amounts of the corresponding ABH substances concordant with the individual's ABO red cell phenotype. Nonsecretors, in contrast, have very little ABH substance, regardless of blood group, in their saliva [10].

In Lewis positive individuals expressing the secretor α1,2 fucosyltransferase (secretors) most of the type 1 precursor is modified into H type 1, which can then be transformed into Le^b by the Lewis α1,3/4 fucosyltransferase or into

* To whom correspondence should be addressed.

related A and/or B structures by ABO and Lewis transferases (e.g. A type 1 and ALe^b). In Lewis positive individuals not expressing the secretor transferase (nonsecretors) the type 1 antigen is only modified by the Lewis transferase, and Le^a antigen results. As a consequence of these interactions and the passive adsorption of glycolipids into the red cell membrane, the red cells of Lewis-positive nonsecretors phenotype as Le(a+b−) while red cells of Lewis-positive secretors phenotype as Le(a−b+). The Lewis and secretor systems at the phenotypic level in some populations, in particular Polynesians [11–15], Aborigines [16, 17], and Asians [18] are more complex. In these individuals a further red cell phenotype Le(a+b+) can be frequently found. This Le(a+b+) phenotype and the associated partial ABH secretor phenotype [14], which is virtually absent in Caucasians, is believed to be caused by an inefficient secretor transferase [14, 17].

The present work describes the immunochemistry of Lewis glycolipid expression in the plasma of common Lewis phenotypes and various individuals of rare Le(a+b+) phenotypes and partial secretor phenotypes.

Materials and methods

Samples

Plasma was obtained from healthy individuals either by routine blood donation or plasmapheresis from 16 Polynesians and one Micronesian of various phenotypes, two Le(a+b+) Chinese from Taiwan (plasma supplied by Dr Lin, Taiwan), and a Le(a+b+) Réunion Islander [19]. Plasma from four Caucasians representing the known Caucasian Lewis/secretor phenotypes was used for controls. Red cell and salivary phenotypes were determined using methodology and antisera as previously described [14, 15]. Secretor phenotypes were determined for the Polynesian and Caucasian control samples but were not determined for the Asian and Réunion Islander samples. A brief description of each sample and its corresponding lane on the thin-layer chromatograms is shown in Table 1.

Table 1. Plasma sample descriptions and thin-layer chromatography lane positions. The ABO (monoclonal antiserum) and Lewis (goat antiserum) phenotypes are red cell defined. The secretor phenotypes were determined from saliva with the partial secretor phenotype as previously defined [14].

TLC lane	*Sample code*	*ABO*	*Lewis*	*ABH secretor phenotype*	*Race*[a]
a	057	O	a+b−	nonsecretor	Samoan
b	033	O	a+b−	partial secretor	Maori
c	029	O	a+b+	partial secretor	Niuean
d	031	O	a+b+	partial secretor	Samoan
e	078	O	a+b+	partial secretor	Maori
f	082	O	a+b+	partial secretor	Maori
g	116	O	a+b+	partial secretor	Niuean
h	055	O	a+b+	secretor	Samoan
j	079	O	a+b+	secretor	Samoan
k	120	O	a+b+	secretor	Samoan
l	LC3	O	a+b+	not determined	Asian
m	LC4	O	a+b+	not determined	Asian
n	021	O	a−b+	secretor	Samoan
o	113	O	a−b+	secretor	$\frac{1}{4}$ Maori
p	122	O	a−b+	secretor	Tongan
q	124	A_1	a−b+	secretor	Samoan
r	126	A_1	a−b+	secretor	$\frac{3}{4}$ Samoan[b]
s	127	A_1	a−b+	secretor	$\frac{3}{4}$ Samoan[b]
t	115	A_2	a−b+	secretor	Nauru Islander
v	Web	O	a−b−	nonsecretor	Caucasian
w	Bli	O	a−b−	secretor	Caucasian
x	Hen	O	a+b−	nonsecretor	Caucasian
y	Fou	O	a+b+	not determined	Réunion Islander
z	Did	O	a−b+	secretor	Caucasian

[a] Maori, Samoan, Niuean, Tongan are all Polynesian races, while the Nauru Islander sample is Micronesian. Where a partial race is indicated the other ancestry is Caucasian.
[b] Siblings.

Glycolipid analysis

Total nonacid glycolipids were prepared as previously described [20]. Approximately 6 µg of total nonacid glycolipids per lane were loaded on to high-performance silica gel thin-layer plates and separated by thin-layer chromatography (TLC) in a solvent system of chloroform: methanol:water, ratio 60:35:8 v/v. Plates were dried and re-eluted in fresh solvent. Glycolipids separated on TLC plates (Merck, Darmstadt, Germany) were analysed by staining with an anisaldehyde reagent [20]. Glycolipids separated on TLC plates (Whatman Ltd, England) were labelled with antibodies in the chromatogram binding assay (CBA) using a modification of the method of Magnani [21, 22].

Glycolipids were identified on the basis of their reactivity with defined reagents and known chromatographic mobilities. The nomenclature used for the glycolipids is the epitope name, and when required or known it is followed by the number of sugar units and then the chain type (if any variations exist) e.g. H-5-1 is a 5 sugar H structure based on a type 1 chain (also known as Le^d), whereas Le^a-5 is a 5 sugar Le^a structure.

Monoclonal antibodies used in the TLC-CBA technique were: anti-Le^a 069 (clone BRIC 87) from South West Regional Transfusion Centre, Southmead, Bristol, UK; anti-Le^{ab} 073 (clone LM129/181), anti-Le^{abH} 074 (clone LM129/180), and anti-Le^{bH} 075 (clone LM137/276) from Glasgow and West of Scotland Blood Transfusion Service Law Hospital, Carluke UK. The immunological and serological properties of these reagents have been described in detail elsewhere [15, 23, 24].

Results

Separate TLC experiments for each Lewis phenotype were run, developed, and photographs from each autoradiograph were then cut and clustered according to Table 1. Only one set of controls (from the Le(a+b+) experiment) are shown (lanes v–z). The triple banding patterns seen in the thin layer experiments are typical for plasma glycolipids and are due to ceramide heterogeneity.

Anisaldehyde chemical staining

As expected of plasma glycolipid extracts the predominating glycolipids were those with 4 or less sugars, although minor bands representing the different blood group glycolipids were seen in the 5–7 sugar regions (results not shown). There was some evidence of elongated structures but the reactions were very weak, indicating only trace amounts of these glycolipids were present.

Thin-layer chromatography-chromatogram binding assays (TLC-CBA)

Antiserum 069 anti-Le^a reacts with the Le^a epitope, cross-reacts with the type 1 precursor, but does not crossreact with the Le^b epitope [23]. This antiserum (Fig. 1A) reacted strongly as expected in the 5 sugar region of the Le(a+b−) control (lane x) clearly identifying Le^a-5. It also reacted in a similar position with all samples with a serologically detectable red cell Le^a antigen (lanes a–m, x and y). Reactivity to a much lesser extent could be found in some of the Le(a−b+) samples (lanes p–t) as expected. Traces of Le^a-5 were, as expected, detected in Le(a−b−) non-secretor sample (lane v) [24]. Reactivity of extended structures (about 9–10 sugars) is evident in some of the Polynesian samples (lanes a, e, f and k) and the Le(a+b−) control (lane x). The Le(a+b+) Réunion Island sample (lane y) reacted in a similar way to the other Le(a+b+) samples (lanes c–m), albeit weaker and with one longer chain ceramide species (faster migration by TLC) predominating.

Antiserum 073 anti-Le^{ab} reacts with both Le^a and Le^b epitopes but does not react with Le^x or Le^y [23]. This antiserum (Fig. 1B) reacted strongly and clearly identified Le^a-5 and Le^b-6 separately in the controls (lanes x and z). Reactivity was found in both the 5 and 6 sugar regions of the two Le(a+b−) Polynesian samples (lanes a and b) and all the Le(a+b+) samples (lanes c–m, and y) showing both Le^a and Le^b to be present. The immunoreactivity patterns of the two Le(a+b−) samples (lanes a and b) are identical to those of the Le(a+b+) samples, suggesting that these samples are in fact Le(a+b+) but with serologically undetectable Le^b. The Caucasian Lewis positive control samples (lanes x and z) do not show this pattern of reactivity. There was also clear evidence of extended structures, particularly in the Polynesian Le(a+b−) and Le(a+b+) samples, although extended structures are also seen in the Caucasian samples (lanes x and z). As expected trace Le^b-6 activity can be seen in the Le(a−b−) secretor (lane w) [24]. The Le(a+b+) Réunion Island sample (lane y) reacted with a similar pattern as the Polynesian Le(a+b+) samples although, in relation to the Le^a reaction the Le^b reaction was much stronger. Once again in this sample it could be seen that Le^a-5 with a longer ceramide chain dominated.

Antiserum 074 anti-Le^{abH} reacts with the Le^b epitope but also shows crossreactivity with Le^a and H type 1 epitopes [23]. This antiserum was essentially unreactive with Le^a-5 and H-5-1 glycolipids as can be seen for the Le(a+b−) (lane x) and Le(a−b−) secretor (lane w) controls, presumably because cross-reactivity is not always seen when working with glycolipids on TLC plates. This antiserum reacted strongly with Le^b-6 (Fig. 1C) in all samples except the Le(a−b−) nonsecretor (lane v) and the Le(a+b−) sample (lane x). Again it is clear that the two Le(a+b−) phenotyping Polynesian samples (lanes a and b) are similar to the Le(a+b+) samples (lanes c–m, and y). Evidence of extended structures is seen in the 8–10 sugar region and in the greater than 10 sugar region for most samples. There does not appear to be much difference in the extended structures between the group O Le(a−b+) (lanes n–p) and the Le(a+b+) samples. The Caucasian Le(a−b+) sample (lane z) was not dissimilar to some of the Polynesian samples. Trace Le^b-6 was detected in the Le(a−b−) secretor control (lane w) as expected [24]. Using this antiserum there appears on the whole to be less Le^b-6 in the Le(a+b+) samples (lanes c–m) than there is in the Le(a−b+) samples (lanes n–t). The Le(a+b+) Réunion Island sample (lane y) reacted with a similar pattern to some of the Polynesian Le(a+b+) samples.

Antiserum 075 anti-Le^{bH} reacts with Le^b and related H epitopes but does not react with the Le^a epitope [23]. This antiserum, despite its reactivity with the Le^b antigen, is useful in its ability to define the H type 1 antigen. This antiserum (Fig. 1D) reacted strongly with Le^b-6 in all samples except the Le(a−b−) nonsecretor (lane v) and the Le(a+b−) sample (lane x), which is in agreement with the reactivity seen with antisera 074 (Fig. 1C). No interpretation on reaction strength between phenotypes should be made from this plate because of experimental differences in staining (as determined from controls, not shown). As expected H-5-1 reactivity is seen only in the Le(a−b−) secretor sample (lane w) and trace amounts of Le^b-6 were also detected. The absence of H-5-1 from all other samples suggests its complete utilization in the formation of Le^b. Glycolipids from selected samples (those in lanes b, e, w, x and y) were also tested against a range of 10 antisera reactive against the H type 2 antigen (not shown). No reactivity was found with the plasma glycolipids indicating an absence of H type 2 structures in these samples and suggesting that the observed reactivity is due to H type 1 and/or Le^b structures.

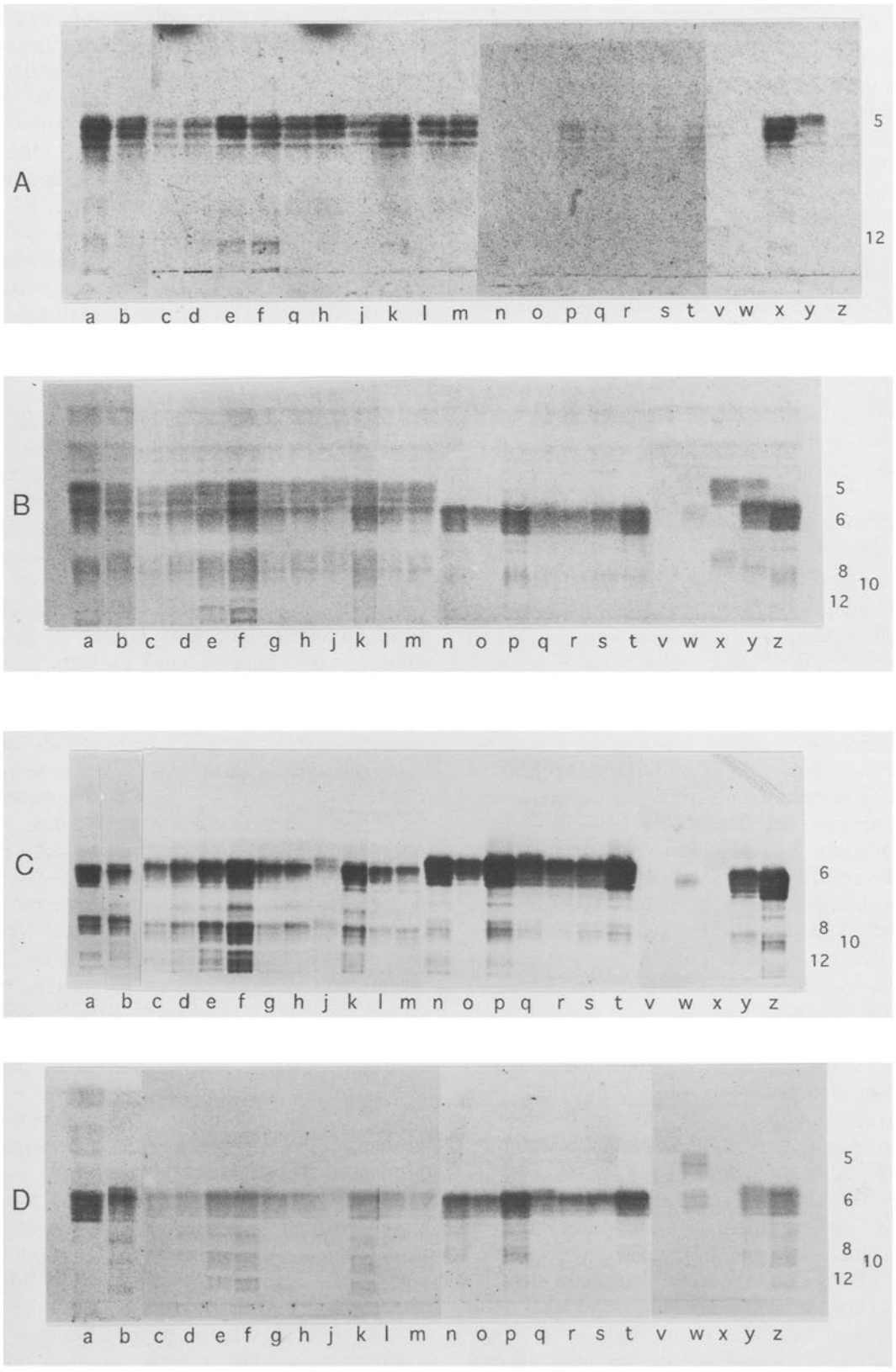

Figure 1. Monoclonal anti-Lewis TLC-CBAs of Lewis positive plasma nonacid glycolipids. Lane positions correspond to sample descriptions in Table 1. Plate A = 069 anti-Le^a, plate B = 073 anti-Le^{ab}, plate C = 074 anti-Le^{abH}, plate D = 075 anti-Le^{bH}. Markings on the right of the plates indicate the number of sugar residues in each band. Migratory positions of larger glycolipids are much more variable and the positions indicated are only approximate.

Discussion

The Le(a+b+) phenotype and associated partial secretory phenotypes are absent or rare in adult Caucasian populations [25] but they are frequent in Polynesians and Asians [11, 14, 17, 18]. Although the exact biochemical basis for these aberrant phenotypes has not yet been resolved, evidence is strongly in support of an inefficient secretor fucosyltransferase, Se^w, while the alternative possibility of a strong *Le*-gene encoded fucosyltransferase has been previously eliminated [14, 15]. In order to extend and support initial observations made on a few selected samples [15], plasma, the source of red cell Lewis antigens, was collected from a spectrum of individuals with unusual Lewis/secretor phenotypes. Samples were obtained from: a Le(a+b−) ABH nonsecretor who secreted Lewis substances; a Le(a+b−) partial secretor; Le(a+b+) partial secretors; Le(a+b+) secretors; and a full range of normal Lewis/secretor phenotypes as controls. The Le(a+b+) samples represented Polynesian, Asian and Réunion Island ethnic backgrounds. Results on the expression of Lewis antigens in Lewis negative individuals have been published elsewhere [24].

Expression of Lewis antigens in the Le(a+b+) and Le(a+b−) phenotypes

At the red cell serological level the Le(a+b+) phenotype is easily recognized although polyclonal and monoclonal anti-Le^b antisera are often variable in their ability to detect the Le^b epitope on Polynesian and Oriental red cells [12, 13, 26]. Reactions with Lewis antisera are usually equally strong, although a whole range of reactions exist.

In Polynesian individuals of the Le(a+b+) phenotype both Le^a-5 and Le^b-6 glycolipids were clearly demonstrated in both plasma (Fig. 1) and red cell glycolipid preparations [15]. The same result was obtained for a Réunion Islander and two Asian plasma samples of the Le(a+b+) phenotype clearly showing that there are no apparent racial differences in glycolipid expression between Le(a+b+) individuals of these races. The copresence of Le^a and Le^b in Le(a+b+) samples is in marked contrast to Caucasians with 'normal' Lewis phenotypes who have either Le^a or Le^b present (although trace amounts of Le^a are, as expected, seen in the plasma of Le(a−b+) individuals [reviewed in 1, 10]). There were no observable differences in glycolipid expression between the Le(a+b+) partial secretor and Le(a+b+) secretor samples suggesting that only minor differences exist between these two different phenotypes.

The Le(a+b−) phenotype in Caucasians is usually clear and easily defined but in other populations where the Le(a+b+) phenotype is common, it appears that the Le^b reaction of some Le(a+b+) phenotype cells may have become so weak as to be undetected by many reagents [13]. We were able to confirm this by demonstrating Le^b glycolipids in both of the Polynesian Le(a+b−) samples, one of which is an apparent nonsecretor and the other a partial secretor. The patterns of reactivity found resembled those of the Le(a+b+) samples with both Le^a and Le^b co-expressed and were unlike those of the Caucasian Le(a+b−) phenotype (Fig. 1). It is therefore more appropriate to designate these cells as being Le(a+b+), although in routine serology it would be unlikely that these red cells would receive this designation. This observation supports the previous suggestion [12, 14, 15] that despite an apparent Le(a+b−) frequency of up to 26% in Polynesians [11] the nonsecretor gene (*se*) is probably either absent or at least very rare in Polynesians.

Despite the different red cell serological profiles between the Polynesian Le(a+b−) samples and the Le(a+b+) samples no plasma glycolipid differences were evident. This suggests that only minor, and probably only quantitative differences between these phenotypes exist, and that serological detection of red cell Lewis antigens may be very sensitive to the level of Lewis glycolipids in plasma. It is alternatively possible that Polynesian Le(a+b−) phenotyping cells have absorbed less Le^b glycolipids from the plasma than the Le(a+b+) phenotyping cells. This is, however, not supported by the parallel observation that these individuals have less salivary substances than the other Le(a+b+) individuals [14]. It therefore appears more likely that the red cells which phenotype as Le(a+b−), but are truly Le(a+b+) with poorly expressed Le^b, result from the action of a *very* weak secretor transferase and therefore simply represent a shift further in favour of the Le transferase. This suggests that there may be a range of the Se^w transferases in different individuals, possibly due to different penetrance or several variants of the *Se* gene. When the Se^w transferase is very inefficient the individual phenotypes as Le(a+b−) with negative to partial secretion of salivary ABH substances, however when the Se^w transferase is more efficient (but still much less so than Se) the red cells phenotype as Le(a+b+) with partial secretion. An even more efficient Se^w transferase will result in Le(a+b+) secretors and possibly Le(a−b+) secretors.

Elongated Lewis reactive structures in Lewis positives

Further complexities in the Polynesian Lewis system are evident when the more slow-moving glycolipids seen on TLC-CBA analysis of the plasma samples are considered. These structures which bear Lewis epitopes on longer and/or more complex saccharide chains appear to be more predominant in the Polynesian samples, although they are noted to a lesser extent in the Caucasian samples, where they are relatively minor glycolipids. There also appears to be variation in the extended structures between individuals (cf. Fig. 1, lanes f and g); however it is difficult to make quantitative interpretations of TLC because of experimental limitations. This observation is supported by evidence from glycolipids isolated from human intestinal epithelial cells. It was found that of the glycolipids with more than 4 sugars

the amount, by weight, of extended glycolipids found in the Le(a+b+) individual was about 25% but only about 10% in the Le(a−b+) individual [27].

The formation of the extended glycolipids in the Le(a+b+) phenotype is compatible with the concept of an inefficient Se fucosyltransferase, i.e. a weak secretor gene. Reduced fucosyltransferase competition offered by the product of a Se^w gene may lead to only partial consumption of the type 1 precursor, thereby allowing for increased production of Le^a and elongation of precursor chains before addition of the α1,2fucosyl residues. The presence of a Se^w gene may therefore explain the increased extended Lewis reactive glycolipids in Polynesians. The addition of α1,4 fucose by the Lewis fucosyltransferase to the subterminal GlcNAc of the precursor will also inhibit chain elongation. However, the Lewis fucosyltransferase appears to be relatively less efficient than the secretor fucosyltransferase in effecting fucosylation of the precursor. This can be seen in the relatively small amount of Le^a glycolipids made in individuals with both Lewis and secretor fucosyltransferases. In this respect the secretor fucosyltransferase is probably more important than the Lewis fucosyltransferase in controlling precursor chain elongation, although the contribution of other factors and glycosyltransferases are unknown.

The contribution of the extended Lewis structures found in Le(a+b+) individuals to red cell phenotyping results is uncertain. The finding of Le(a+b+) cells serologically phenotyping as Le(a+b−) yet appearing to have similar extended Lewis structures to the Le(a+b+) phenotyping cells suggests that the involvement of these extended structures in phenotyping is at best minimal. It might be that extended glycolipids are poorly or not expressed on the red cell membrane, because extended glycolipids, where the carbohydrate moiety dominates over lipid, are more soluble in water and may have a lesser tendency to absorb on to red cells. All the same, quantitative differences in the expression of Le^b antigens appear to be responsible for the serological phenotyping difficulties encountered with some cells.

The significance, if any, of the extended structures raises important questions especially when it is noted that extended type 1 Lewis structures are considered to be tumour-associated antigens [28–30]. The finding here of relatively large quantities of extended glycolipids in healthy individuals, assumed to bear type 1 Lewis epitopes based on their reactivity with monoclonal antibodies, will however have to await structural confirmation. Such work is in progress.

The Le(a+b+) and associated unusual red cell and salivary phenotypes are believed to be caused by a change in the equilibrium of the action of secretor and Lewis gene-specified transferases as caused by a variant weak secretor gene Se^w [14, 15, 17]. The phenotypic result of this changed equilibrium is: Le(a+b+) red cells and partial ABH secretion, or Le(a+b−) red cells and partial to negative ABH secretion, when the shift is further in favour of the *Le* gene [14, 15]. The results here suggest that there is either a variable penetrance of the secretor gene in different individuals, or tissues, or there are several Se^w variants. Until the secretor genes are cloned this issue will remain unresolved. The results shown here are also in support of the concept that reduced secretor fucosyltransferase competition allows for the formation of extended Lewis glycoconjugates [24, 27].

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References

1. Oriol R, Le Pendu J, Mollicone R (1986) *Vox Sang* **51**:161–71.
2. Clausen H, Hakomori S (1989) *Vox Sang* **56**:1–20.
3. Mourant AE (1946) *Nature* **158**:237–8.
4. Andresen PH (1948) *Acta Path Microbiol Scand* **25**:728.
5. Marcus DM, Cass LE (1969) *Science* **164**:553–55.
6. Oriol R, Danilovs J, Lemieux R, Terasaki P, Bernoco D (1980) *Hum Immunol* **3**:195–205.
7. Dunstan RA, Marcus B, Simpson MD, Rosse WF (1985) *Am J Clin Pathol* **83**:90–94.
8. Grubb R (1948) *Nature* **162**:933.
9. Brendemoen OJ (1950) *J Med Clin* **36**:335–41.
10. Race RR, Sanger R (1975) In *Blood Groups in Man*, 6th ed.: pp. 311–22. Oxford: Blackwell Scientific Publications.
11. Henry SM, Simpson LA, Woodfield DG (1988) *Hum Hered* **38**:111–16.
12. Henry SM, Simpson LA, Benny AG, Woodfield DG (1989) *NZ J Med Lab Technol* **43**:64–67.
13. Henry SM, Dent AM, Harding Y (1990) In *Proceedings of the second international workshop and symposium on monoclonal antibodies against human red blood cells and related antigens.* Lund, p. 75.
14. Henry SM, Benny AG, Woodfield DG (1990) *Vox Sang* **58**:61–66.
15. Henry SM, Woodfield DG, Samuelsson BE, Oriol R (1993) *Vox Sang* **65**:62–69.
16. Vos GH, Comley P (1967) *Acta Genet* **17**:495–510.

17. Sturgeon P, Arcilla MB (1970) *Vox Sang* **18**:301–22.
18. Lin-Chu M, Broadberry RE, Chang FJ (1988) *Transfusion* **28**:350–52.
19. Ventura M, Gibaud A, Le Pendu J, Hillaire D, Gérard G, Vitrac D, Oriol R (1988) *Hum Hered* **38**:36–43.
20. Karlsson KA (1987) *Methods Enzymol* **138**:212–20.
21. Magnani JL, Smith DF, Ginsburg V (1981) *Anal Biochem* **109**:399–402.
22. Hansson GC, Karlsson KA, Larson G, Samuelsson BE, Thurin J, Bjursten LM (1985) *J Immunol Methods* **83**:37–42.
23. Good AH, Yau O, Lamontagne LR, Oriol R (1992) *Vox Sang* **62**:180–89.
24. Henry SM, Oriol R, Samuelsson BE (1994) *Vox Sang* (in press).
25. Mourant AE, Kopec A, Domaniewska-Sobczak K (1976) In *The Distribution of the Human Blood Groups and Other Polymorphisms*, 2nd ed.: pp. 548–76. Oxford: Oxford University Press.
26. Lin-Chu M, Broadberry RE (1990) In *Proceedings of the second international workshop and symposium on monoclonal antibodies against human red blood cells and related antigens.* Lund: p. 79.
27. Henry SM, Samuelsson B, Oriol R (1994) *Glycoconjugate J* **11**:600–7.
28. Stroud MR, Levery SB, Nudelman ED, Salyan MEK, Towell JA, Roberts CE, Watanabe M, Hakomori S (1991) *J Biol Chem* **266**:8439–46.
29. Stroud MR, Levery SB, Salyan MEK, Roberts CE, Hakomori S (1992) *Eur J Biochem* **203**:577–86.
30. Watanabe M, Ohishi T, Kuzuoka M, Nudelman ED, Stroud MR, Kubota T, Kodaira S, Abe O, Hirohashi S, Shimosata Y, Hakomori S (1991) *Cancer Res* **51**:2199–204.