Differential Tissue Expression of the Lewis Blood Group Antigens: Enzymatic, Immunohistologic, and Immunochemical Evidence for Lewis a and b Antigen Expression in Le(a-b-) Individuals

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The Lewis blood group system comprises two main carbohydrate antigens, Le<sup>a</sup> and Le<sup>b</sup>. Lewis typing has traditionally been based on serologic determinations using erythrocytes and saliva. Several recent studies have demonstrated that erythrocyte Lewis phenotype may change during pregnancy or disease, and inappropriate Lewis antigens have been found in both normal and neoplastic tissue. To evaluate whether these observations are in conflict with the presently proposed genetic and biosynthetic basis of the Lewis blood group system, we performed a combined enzymatic, immunohistologic, and immunochemical study of Lewis antigen expression in normal and neoplastic tissues, as well as erythrocytes, plasma, and saliva of Le(a-b-) typed individuals.

In this study, enzymatic, immunohistologic, and immunochemical methods were used to gain insight into expression of Lewis a and b antigens and corresponding α1 → 4FT activities in individuals typed as Le(a-b-) by standard hemagglutination. Owing to the rare occurrence of Le(a-b-) individuals (~6% of the white population), only six individuals were examined. Two groups of Le(a-b-) RBC-typed individuals were identified. One group (consisting of three individuals) was classified as nongenuine Le(a-b-) (α1 → 4FT activity detected in saliva); the other (also consisting of three individuals) was classified as genuine Le(a-b-) (no α1 → 4FT activity detected in saliva). The nongenuine Le(a-b-) individuals had chemical amounts of Lewis active neutral glycolipids in their RBC and serum similar to Lewis' individuals, regardless of lack of hemagglutination. In the genuine Le(a-b-) group, none or very small quantities of Lewis a and b antigens were detected in RBC, plasma, and saliva. Of six cancer-bearing patients typed Le(a-b-), three were identified as nongenuine owing to the presence of α1 → 4FT activity and Lewis antigens in saliva and three were identified as genuine (lacking α1 → 4FT and Lewis antigens in saliva). These genuine Le(a-b-) individuals were shown to express significant α1 → 4FT in tissues, and Lewis antigens were detected in tissues by immunohistology as well as immunochemistry. We conclude that the Lewis phenotype obtained by serologic determination of erythrocytes and saliva does not apply to all tissues. We discuss biosynthetic and genetic consequences of this finding.
found on RBC or in serum, whereas Lewis antigens and low but readily detectable α1-→4FT activity were found in colon and bladder tissues.

MATERIALS AND METHODS

Samples. Fifty-milliliter samples of human whole blood were obtained from six individuals by venipuncture, partly used within 24 hours for serology, and partly centrifuged and stored at -80°C as serum and cells. Saliva was obtained simultaneously with the blood samples, and 1 mL was boiled and stored at -20°C for hemagglutination inhibition studies. Biopsies were taken from colon tumors as well as serum and cells. Saliva was obtained simultaneously with the blood samples, and 1 mL was stored at -80°C for enzyme analysis.

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FT assays on saliva. Saliva was thawed and frozen twice and centrifuged at 800g, and the supernatant was used for assays. For assays of α1 → 3FT and α1 → 4FT, 20 μL enzyme source was added to GDP-L-[*~C]-FUC (0.28 nmol, 70,000 cpm), MnCl₂ (1 pmol), acceptor (0.5 pmol), ATP (0.5 pmol), Triton X-100 (500 μg), and 0.1 mol/L Tris-HCl (pH 7.2) in a total volume of 100 μL. The mixture was incubated for 2 hours and chromatographed on Whatman no. 40 paper in ethyl acetate/pyridine/water (10:4:3 vol/vol/vol) for 48 hours. The papers were dried, scanned for radioactivity (Packard radiomachromatogram scanner, Downersgrove, IL), and the mobility of peaks was measured relative to known compounds; these areas were then cut out and counted by a liquid scintillation counter.

N-Acetyllactosamine (Galβ1 → 4GlcNAc) was used as acceptor for the α1 → 3FT determination, and lacto-N-biose I (Galβ1 → 3GlcNAc) was used as acceptor for the α1 → 4FT. FT assay on tissues. Tissue samples (1 g) were homogenized in 2 vol 50 mmol/L HEPES buffer (pH 7.2), 0.5 mol/L sucrose, and 1 mmol/L EDTA by two strokes of a Potter-Elvehjem homogenizer and used for characterization of enzyme activities present in each fraction. The FT activity was determined as previously described[7] in reaction mixtures containing 2.5 μmol HEPES buffer (pH 7.2), 40 μg lactotetraocyclceramide (Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer) (LC₄) or lacto shootoutraocyclceramide (Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer) (LC₃), 100 μg taurodeoxycholate, 1 μmol MnCl₂, 0.5 μmol CDP-choline, 15 nmol GDP-[¹⁴C]-Fuc (15,000 cpm/mmol), and 100 to 300 μg protein in a total volume of 0.1 mL. The reaction mixture was incubated for 2 hours at 37°C and terminated by addition of 6 pmol EDTA and 0.1 mL chloroform-methanol (CM) 2:1. The entire reaction mixture was streaked onto a 4-cm-wide strip of Whatman no. 3 paper and chromatographed with water overnight. The glycolipid remaining at the origin was extracted with 2- to 5-mL washes of chloroform-methanol-water (CMW) 10:5:1. The solvent was removed by nitrogen stream, and the glycolipid was dissolved in 20 μL CM 2:1. A 10-μL aliquot was removed, spotted onto an high-performance thin-layer chromatography (HPTLC) plate (Merck, Darmstadt, FRG), and developed in CMW 60:5:1 containing 0.02% CaCl₂ as a
final concentration. Standard glycolipids were visualized by orcinol spray. Radioactive glycolipid bands were located by autoradiography, scraped from the plate, and counted by liquid scintillation counter.

Isolation of glycolipids. RBC (\(> 20 \text{ mL}\)) and serum (\(> 30 \text{ mL}\)) were extracted overnight at 4°C with solvent A (isopropanol-hexane-water [IHW] 65:25:20). Tissues were extracted with 10 vol solvent A in a Potter-Elvehjem homogenizer, sonicated, and centrifuged at 800g for 20 minutes. The insoluble pellet was re-extracted twice in an identical way, and the combined supernatants were evaporated under nitrogen stream. The near-dry samples were then dissolved in CMW 30:60:8 and subjected to chromatography on diethylami-noethanol (DEAE) Sephadex A-25\(^\text{a}\) to separate total neutral glycolipids from gangliosides. The total neutral glycolipid fraction was dried and acetylated with 1 mL pyridine and 0.5 mL acetic anhydride, followed by chromatography on a Florisil column and deacetylation with \(\frac{1}{4}\)vol 0.5% sodium methoxide in CM 2:1. The samples were dried and resuspended in CM 2:1, and 5 \(\mu\)L of this total neutral glycolipid fraction was spotted on HPTLC plates, developed in CMW 50:40:10, and stained with orcinol. The amount of glycolipid in each lane was standardized according to the extent of orcinol staining.

**TLC immunostaining.** Immunostaining of glycolipids separated by HPTLC was performed according to the procedure of Magnani et al\(^\text{b}\) as modified by Kannagi et al.\(^\text{a}\) The monoclonal antibodies (MoAbs) used were anti-Le\(^a\) clone CF4-C, (IgG)\(^3\), and anti-Le\(^b\) (IgM) donated by Dr Donald A. Baker (Chembioinert, Alberta, Canada). TLC immunostaining with the anti-Le\(^a\) MoAb produced staining in the hexaosylceramide region. In glycolipid extracts of tissues, staining of the H type 1 pentaosylceramide region was also observed, indicating cross-reactivity with H type 1 structures. Only one anti-Le\(^a\) MoAb was used for TLC immunostaining studies owing to the limited amount of lipid extract available.

**Immunohistochemistry.** Immunohistochemical studies were performed with two additional IgM anti-Le\(^a\) MoAbs: one from Biotest-Serum Institut, GMBH, Frankfurt, FRG, the second donated by L. Messetter, Malm@ Hospital, Sweden. The specificity of the latter MoAb has been studied in detail.\(^\text{9}\) An IgG, MoAb, FH7,\(^\text{16}\) directed to the sialosyl derivative of Le\(^a\) (sialosyl-Le\(^a\)), was also used.

A modification of previously described methods\(^\text{17,18}\) was used. Formalin-fixed, paraffin-embedded 3-\(\mu\)m sections were deparaffinized and rehydrated. Endogenous peroxidase was blocked by 0.8% \(\text{H}_2\text{O}_2\) in absolute methanol for 30 minutes. Sections were washed in Tris/Phosphate-buffered saline (PBS), incubated with 10% normal rabbit serum in PBS for 10 minutes, and then incubated overnight at 4°C with MoAbs diluted 1:40 to 1:80 in PBS. After repeated washings, MoAb binding was visualized by incubation for 60 minutes with biotinylated rabbit anti-mouse immunoglobulins (diluted 1:100 in PBS), followed by avidin-biotin-peroxidase complex method according to the manufacturer's instructions (Dako, Copenhagen, Denmark) and 0.04% 3-amino-9-ethylcarbazole. The three anti-Le\(^a\) MoAbs showed similar staining reactions. MoAb FH7 (antialysyl-Le\(^a\)) stained more cells than the anti-Le\(^a\) MoAb in colonic sections.

**RESULTS**

Genuine Le\(^a\)-(a-b-) individuals. Three individuals were identified as genuine Lewis-negative individuals because their RBC typed Le\(^a\)-(a-b-) and their saliva contained no \(\alpha1\rightarrow4\text{FT}\) activity (Table 2). Normal colon and colon cancer tissue was available from only two of three and in one only bladder cancer tissue was available. Normal colon tissue and bladder tumor tissue from Lewis-positive individuals served as control.

**\(\alpha1\rightarrow4\text{FT}\) activity of tissue from Le\(^a\)-(a-b-) individuals.** In normal colonic mucosa, colon carcinoma tissue, and bladder cancer tissue, \(\alpha1\rightarrow4\text{FT}\) activity was readily detectable with the type-1 chain glycolipid acceptor Le4 (Table 2). The specific activity of the \(\alpha1\rightarrow4\text{FT}\) was similar in all five specimens examined and considerably lower (5% to 16%) than the activity in Lewis-positive controls (Table 2).

In contrast, significant \(\alpha1\rightarrow3\text{Fuc}\) transfer into the type-2 chain glycolipid acceptor NLe4 was observed in all samples regardless of Lewis status of donor. TLC analysis of reaction products from transfer of \(^{14}\text{C-Fuc}\) from GDP-[\(^{14}\text{C}\)]-Fuc to the type-1 chain acceptor Le\(^a\) and the type-2 chain

### Table 2. Serologic, Enzymic, Immunohistologic, and Immunochromatographic Findings in Genuine Le\(^a\)-(a-b-) Individuals

<table>
<thead>
<tr>
<th>Material</th>
<th>Serology*</th>
<th>Enzyme Activity ((\mu\text{mol/h/mg Protein}))</th>
<th>Immunohistology†</th>
<th>HPTLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBCs</td>
<td>Saliva</td>
<td>Tissue†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\alpha1\rightarrow3)</td>
<td>(\alpha1\rightarrow4)</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon Co(_1)</td>
<td>A(_1)</td>
<td>Le(^a)-(a-b-) 2 8 266 90 0 1,990 60 + − + (+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon Co(_2)</td>
<td>A(_1)</td>
<td>Le(^a)-(a-b-) 2 8 266 340 0 1,320 70 + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon Co(_1)</td>
<td>A(_1)</td>
<td>Le(^a)-(a-b-) 2 8 266 90 0 2,000 47 + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon Co(_2)</td>
<td>A(_1)</td>
<td>Le(^a)-(a-b-) 2 8 266 340 0 1,460 177 + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder B(_1)</td>
<td>A(_1)</td>
<td>Le(^a)-(a-b-) 4 8 4 308 0 608 98 − + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewis-positive control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal colon (n = 3)</td>
<td>16 &gt; 266 &gt; 266</td>
<td>581 &gt; 293 &gt; 2,480 &gt; 1,190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer, bladder (n = 5)</td>
<td>16 &gt; 266 &gt; 266</td>
<td>472 &gt; 632</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Hemagglutination with polyclonal sera, Dolichus biflorus, and Ulex europaeus lectins defined RBC phenotype; hemagglutination inhibition was used to determine titer in saliva.

†Saliva was incubated with GDP-[\(^{14}\text{C}\)]-Fuc (0.28 nmol, 70,000 cpm), MnCl\(_2\) (1 \(\mu\)mol), acceptor (0.5 \(\mu\)mol) lacto-N-bisce (\(\alpha1\rightarrow4\text{FT}\)) or \(\alpha1\rightarrow4\text{FT}\) in a total volume of 100 \(\mu\)L.

‡HEPES buffer (pH 7.2) 2.5 \(\mu\)mol, LC, or NLC, 40 \(\mu\)g, taurodeoxycholate 100 \(\mu\)g, MnCl\(_2\) (1 \(\mu\)mol), CDP-choline 0.5 \(\mu\)mol, GDP-[\(^{14}\text{C}\)]-Fuc 15 nmol (15,000 cpm/nmol), and 100 to 300 \(\mu\)g protein in a total volume of 0.1 ml. Material for assay was homogenized in 50 mmol/L HEPES buffer (pH 7.2), 0.5 mol/L sucrose, and 1 mmol/L EDTA.

§Anti-Le\(^a\) and three different anti-Le\(^a\) MoAbs were used to demonstrate Lewis structures on tissue sections and TLC. Similar staining was observed with the three anti-Le\(^a\) MoAbs.
acceptor nLc, was conducted with enzyme fractions from Lewis-positive and genuine Lewis-negative donors. These results are shown in Fig 1. Strong bands corresponding to formation of α1→3Fuc derivatives of nLc were found with either fraction (lanes 2 and 4), as was a product corresponding to α1→2 fucosylation of the terminal Gal of Lc (lanes 1 and 3), most probably associated with the secretor gene status of the donors. Fucose transfer in α1→4 linkage to Lc resulted in a strong band with the Lewis-positive donor (Fig 1, lane 1), which was greatly reduced but still detectable with the genuine Lewis-negative donor (Fig 1, lane 3). Further analysis of the isolated band corresponding to III′FucLc, from the Lewis-negative donor was conducted by TLC analysis of the acetylated derivative.36 The acetylated product comigrated with standard III′FucLc, but not III′FucnLc, indicating that the product contained an α1→4 linked Fuc on Lc (data not shown). The relative intensity of the bands reflects the competition between the α1→4FT and α1→2FT for their mutual acceptor Lc, and that of the α1→3FT and α1→2FT for their mutual acceptor nLc. In the former case, the α1→4FT dominates in Lewis-positive individuals (Fig 1, lane 1), whereas the α1→2FT dominates in Lewis-negative individuals (Fig 1, lane 3). In the latter case, the α1→3FT is so strongly expressed in most individuals as compared with α1→2FT that only insignificant amounts of α1→2-fucosylated products are formed (Fig 1, lanes 2 and 4).

Lewis antigens in tissues of genuine Le(a−b−) individuals. The genuine Le(a−b−) status of the examined individuals was confirmed by the nearly complete absence of Lewis active glycolipids when total neutral glycolipids were extracted from RBC and serum and subjected to immunochemical characterization (Fig 2A). Normal and malignant tissue specimens examined by this method all contained pentasaccharide Leα and hexasaccharide Leβ antigens however (Fig 2A). These findings were further supported by immunohistochemical detection of Lewis antigens on tissue sections (Fig 3) from all five specimens. The presence of small quantities of Leα and Leβ active glycolipids in RBC extracts of the bladder carcinoma patient (Fig 2A, B, samples) is obscure. The migration of the glycolipids, as well as the immunoreactivity, indicates that these are authentic Leα and Leβ glycolipids. This patient had no Leα or Leβ active glycolipids in serum extract.

Nongenuine Le(a−b−) individuals. Three individuals, two with colon cancer and one with a bladder tumor were serologically defined as Le(a−b−) individuals. Detailed study of their saliva showed α1→4FT activity and secretion of Lewis antigens, however; therefore, they are classified as nongenuine Le(a−b−) (Table 3). Normal colon tissue was obtained from the two colon carcinoma patients; from the third patient, bladder carcinoma tissue was available.

FT activity in tissue from non-genuine Le(a−b−) individuals. In the two specimens of normal colon mucosa, an α1→4FT activity similar to that in the Lewis positive control group (Table 2) was detected (Table 3). In the bladder carcinoma, the α1→4FT activity was in the same range as that of genuine Lewis-negative individuals (Table 3). The α1→3FT was active to an extent similar to that of the other individuals examined (Table 3).

Lewis antigens in tissues from nongenuine Le(a−b−) individuals. Immunostaining of extracted total neutral glycolipids showed pentasaccharide Leα and hexasaccharide Leβ antigens in tissues, and interestingly, also in RBC and serum although RBC could not be agglutinated by anti-Lewis sera (Fig 3B). As expected, immunohistochemistry showed Lewis antigens in these individuals (Table 3).

DISCUSSION

Lewis blood group status is traditionally determined by RBC and saliva phenotyping. Based on such phenotyping, we identified three persons as genuine Lewis-negative individuals having Le(a−b−) RBC and no α1→4FT activity in saliva. Three other individuals were identified as nongenuine Le (a−b−) because their RBC were typed Le(a−b−) by serology but their saliva contained α1→4FT activity and Lewis a and b antigens.

In the group of genuine Le(a−b−) individuals, we were able to identify Lewis a and b antigens and detect low α1→4FT activity in colon and bladder tissue, indicating in vivo activity of the α1→4FT. In the group of nongenuine Le(a−b−) individuals, an α1→4FT activity corresponding to that detected in Lewis-positive individuals, as well as Lewis antigens, were detected in colon and bladder. Owing to great individual variation in enzyme activity, homozygous and heterozygous individuals cannot be separated, but the relatively high frequency of heterozygous (Le, le; le, Le) individuals in the population makes it unlikely that the relatively rare nongenuine Le(a−b−) individuals should simply be the heterozygous individuals.
The demonstration of small quantities of Le\(^a\) and Le\(^b\) antigens on RBCs, but not serum, of a bladder carcinoma patient classified as genuine Le\((a-b-))\) raises the possibility that the antigen originates from Lewis a and b antigen-positive tumors. They may therefore constitute authentic "tumor markers" similar to those observed for sialosyl derivatives of Le\(^a\) antigen.\(^{12,40}\) In the case of glycolipids, these may be more concentrated on RBC as compared with
Table 3. Serologic, Enzyme, Immunohistochemical, and Immunofluorescence Findings in Nonneutropenic Leu(-) B-lymphocyte (Lymphoma, Myeloma, etc.)

<table>
<thead>
<tr>
<th>Material</th>
<th>ABC</th>
<th>Dual</th>
<th>LSC</th>
<th>Monoclonal Antibody</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
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<td></td>
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<tr>
<td>Plasma</td>
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</table>

Other investigations have reported the existence of Leu(-) B-lymphocytes, which have been identified by their expression of Leu-2, Leu-4, Leu-6, and Leu-8 antigens in the blood of Leu(-) B-lymphocytes and in lymph node tissue. In a recent study of blood of patients with Leu(-) B-lymphocytes, a similar B-lymphoma was found in the bone marrow of Leu(-) B-lymphocytes, which suggests the existence of Leu(-) B-lymphocytes in lymph node tissue.

![Image of immunohistochemical staining](image-url)

Legend:
- **ABC**: Anti-B-Cell
- **Dual**: Dual Antibody
- **LSC**: Lymphocyte Surface Carbohydrate
- **Monoclonal Antibody**: Monoclonal antibody
- **Clone**: Clone number

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patients may have Lewis antigens in tissues or secretions, because they probably are genetically identical to Lewis-positive individuals. Owing to some unknown rearrangement of their RBC membrane, however, their RBC cannot agglutinate with anti-Lewis sera although chemically they contain detectable quantities of Lewis active glycolipids. The three persons we identified as nongenuine Le(a-b-) individuals all had terminal cancer. This is in accordance with previous reports of occurrence of a RBC Le(a-b-) phenotype in individuals previously typed as Lewis positive in association with changed biologic conditions such as pregnancy, alcoholic cirrhosis and pancreatitis, hydatid cysts, and various carcinomas.

The finding of α1→4FT activity leading to formation of Lewis antigens in vivo in genuine Le (a-b-) individuals is of interest because such persons are assumed to be homozygous for an inactive allele le at the Lewis gene locus. The operational definition for Lewis-positive and Lewis-negative individuals does not specify any mechanism for the observed differential expression, however. In view of our results, the distinction between Lewis-positive and Lewis-negative individuals does not appear to be qualitative and alternate mechanisms for differential expression might be proposed as follows.

First, the present findings may reflect differing tissue distribution of the Lewis FT in Lewis a or b antigen-positive individuals as compared with Lewis a or b antigen-negative individuals, resulting in the absence of antigen activity in saliva but presence of antigen activity in certain tissues. This is consistent with results from human colon in which high α1→2FT activity was observed in mucosa and low activity of the same transference was observed in rectum, with results from bladder tissue showing synthesis of Leb+ from different tissues. Biochem Biophys Res Commun 260:7619, 1985.

Second, the observed pattern of expression may indicate the existence of a hitherto-unrecognized α1→4FT not associated with the Lewis gene. Such an enzyme may be detectable only in Lewis-negative individuals, however, in whom it is not masked by the Lewis enzyme. The α1→2 FTs are examples of similar transferases that are coded by two different structural genes.

Third, a probably more likely alternative involves an analogy to the blood group A subgroups. Some members of such subgroups inherit an A gene coding for an A transferase with high acceptor affinity (A, transferase), whereas others inherit a gene coding for an enzyme with lower acceptor affinity and more restricted substrate specificity (A, transferase), yielding different levels of cell surface A antigen characterizing the phenotype. Although current knowledge regarding number and specificities of α1→2FTs is ambiguous, α1→3 transfer apparently is ascribable to the same protein, based on kinetic data. In addition, in recent studies using gene expression data in which human DNA was transfected into COS-1 cells, an enzyme with both α1→3(N-acetyllactosamine as acceptor) and α1→4FT activity was identified and expressed (John B. Lowe, personal communication, March 1990). By similar techniques, an α1→3FT activity with no α1→4FT activity was recently identified. If the number of FT genes is limited to the presently identified Lewis (α1→2FT) and “X” (α1→3FT), the Lewis gene probably comprises several variants similar to the blood group A genes, with differing relative reactivities to type-1 and type-2 chain acceptors.

The specific mechanism for the expression of Lewis a and b antigens in Lewis-negative individuals is complex and still unresolved. Although a few individuals were studied, however, we conclude that RBC and saliva Lewis blood group phenotype do not provide complete information regarding the Lewis phenotype in epithelia in general, most likely owing to Lewis gene-encoded transferase proteins with variable activity.

REFERENCES

2. Kumaizaki T, Yoshida A: Biochemical evidence that secretor gene, Se, is a structural gene encoding a specific fucosyltransferase. Proc Natl Acad Sci USA 81:4193, 1984

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of a serologically active fucose-containing trisaccharide from hu-
mans and globin group Le" substance. Nature 204:740, 1964
14. Marr AMS, Donald ASR, Watkins WM, Morgan WTJ: Mo-
ler genetic aspects of human blood group Le" specific-
15. Grollman EF, Kobata A, Ginsburg V: An enzymatic basis for
16. Watkins WM: Biochemistry and genetics of the ABO, Lewis,
and P blood group systems, in Harris H, Hirschhom K (eds):
Advances in human genetics, vol 10, New York, NY, Plenum Press,
1980, p 1
17. Bjork S, Breimer ME, Hansson GC, Karlsson KA, Leffler H:
Structures of blood group glycosphingolipids of human small
18. Sakamoto J, Yin BWT, Lloyd KO: Analysis of the expression
of Lewis, X, and precursor blood group determinants in sali-
va and red cells using a panel of mouse monoclonal antibodies.
Mol Immunol 21:1093, 1984
19. Limas C: Detection of urothelial Lewis antigens with mono-
20. Hirano K, Kawa S, Oguchi H, Kobayashi T, Yonekuni H,
Ogata H, Homma T: Loss of Lewis antigen expression on erythro-
Sears H, Steplewski Z, Koprowski H: Monoclonal antibody localiza-
22. Stigendal L, Olsson R, Rydberg L, Samuelsson BE: Blood
group Lewis phenotype on erythrocytes and in saliva in alcoho-
23. Makni S, Dalix AM, Caillard T, Compagnon B, Le Pendu J,
Aved K, Oriol R: Discordance between red cell and saliva Lewis
24. Pipps RF, Perry PM: Lewis negative genotype and breast
cancer risk. Lancet 1:1198, 1989
25. Paturak NL, King MC, Dupuy ME: Lewis negative genotype
26. Rowe GP, McGregor M, Napier JAF: Lewis negative genotype
27. Òrntoft TF, Wolf H, Watkins WM: Activity of the human
blood group ABO, Se, H, Le, and X gene-encoded glycosyltrans-
fases in normal and malignant bladder urothelium. Cancer Res
48:4427, 1988
28. Holmes EH, Hakomori S, Ostrander GK: Synthesis of type 1
and 2 lacto series glycolipids in human colon adenocarcinoma
and derived cell lines is due to activation of a normally unex-
pressed \( \beta \rightarrow 3\)-acetylglucosaminyl-transferase. J Biol Chem
262:15649, 1987
29. Yu RK, Ledeen RW: Gangliosides of human, bovine, and
30. Magnani JL, Smith DF, Ginsburg V: Detection of ganglio-
sides that bind cholera toxin: Direct binding of \( ^{131} \)-labeled toxin to thin-layer chromatograms. Anal Biochem 109:399, 1980
31. Kannagi R, Nudelman E, Levery SB, Hakomori S: A series of
human erythrocyte glycosphingolipids reacting to the mon-
oclonal antibody directed to a developmentally regulated antigen,
32. Young WW Jr, Johnson HS, Tamura Y, Karlsson K-A,
Larson G, Parker JMR, Khare DP, Sphor U, Baker DA, Hinds-
gaul O, Lemieux RU: Characterization of monoclonal antibodies spe-
cific for the Lewis A human blood group determinant. J Biol Chem
258:4890, 1983
33. Messelet L, Brodin T, Chester MA, Karlsson K-A, Zopf D,
Lundblad A: Immunochemical characterization of a monoclonal
anti-Le" blood grouping reagent. Vox Sang 46:66, 1984
34. Nudelman E, Fukushima Y, Levery SB, Higuchi T, Hakomori S:
Novel fuco-oligosaccharides of human adenocarcinoma: Disialyl
Le" antigen (III14FucII16NeuAc-IV3NeuAcLc,) of human colonic aden-
carcinoma and the monoclonal antibody (FH7) defining this
35. Hsu S-M, Raine L, Fanger H: Use of avidin-biotin-
peroxidase complex (ABC) in immunoperoxidase techniques: A
comparison between ABC and unlabeled antibody (PAP) proce-
36. Òrntoft TF, Wolf H, Clausen H, Hakomori S, Dabelsteen E:
Blood group ABO and Lewis antigens in fetal and normal adult
blood urothelium: Immunohistochemical study of type 1 chain
37. Saito T, Hakomori S: Quantitative isolation of total gly-
38. Magnani JL, Levery SB, Higuchi T, Hakomori S: Discrimina-
39. Òrntoft TF, Wolf H: Blood group ABO and Lewis antigens
in bladder tumors: Correlation between glycosyltransferase activity
Suppl 4:126, 1988
40. Paulson JC, Weinstein J, Schauer A: Tissue-specific expres-
41. Schachter H, Michaels MA, Tilley CA, Crookston MC,
Crookston JH: Qualitative differences in the N-acetyl-D-galac-
tosaminyltransferases produced by human A' and A" genes. Proc
Natl Acad Sci USA 70:220, 1973
42. Clausen H, Hakomori S: Novel blood group H
glycolipid antigens exclusively expressed in blood group A and AB
erthrocytes (type 3 chain H): II. Differential conversion of
different H substrates by A, and A' enzymes, and type 3 chain H
expression in relation to secretor status. J Biol Chem 261:1388,
1986
43. Watkins WM, Greenwell P, Yates AD, Johnson PH: Regu-
lation of expression of carbohydrate blood group antigens. Biochimie
70:1597, 1988
44. Stanley P, Kumar R, Potvin B, Howard D: Use of CHO
glycosylation mutants to clone mammalian glycosyltransferases, in
Sharon N, Liss H, Duksin D, Kahane I (eds): Proceedings of the
Xth International Symposium on Glycoconjugates, Jerusalem,
Israel, 1989, (abstr 54)
Differential tissue expression of the Lewis blood group antigens: enzymatic, immunohistologic, and immunochemical evidence for Lewis a and b antigen expression in Le(a-b-) individuals

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