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Immunochemical and immunohistological expression of Lewis histo-blood group antigens in small intestine including individuals of the Le(a+b+) and Le(a−b−) nonsecretor phenotypes

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Histological samples and total non-acid glycosphingolipids were prepared from small intestine of human cadavers with the Le(a+b+) and Le(a−b−) nonsecretor phenotypes and contrasted with the more common Lewis phenotypes. Glycolipid fractions were analysed by thin-layer chromatography and tested for Lewis activity with monoclonal antibodies reactive to Lewis epitopes. Paraffin-embedded small intestine sections were also fluorescently immunostained with anti-Lewis antibodies. Unlike the common Lewis positive phenotypes, we were immunochemically able to demonstrate the copresence of large amounts of Le^a and Le^b glycolipids in the Le(a+b+) sample. In addition we demonstrated increased formation of extended Lewis structures in this phenotype. By immunohistochemistry Le^a, Le^b and type 1 precursor chain epitopes could be demonstrated in the brush border. These results show that the expression of the Le(a+b+) phenotype at the erythrocyte phenotyping level parallels the small intestinal expression of this phenotype, and the patterns of Lewis antigen expressions are unique to this phenotype. By immunohistochemistry and immunochemistry we also demonstrated the presence of trace amounts of Lewis active glycoconjugates in the small intestine of the Le(a−b−) nonsecretor and Le(a+b−) samples. In the Le(a−b−) nonsecretor Le^a and Le^b activity was absent and type 1 precursor was present in brush border, while Le^b activity was immunohistologically demonstrated in the Golgi apparatus of the deep glands. Trace amounts of both Le^a and Le^b glycolipids were identified in this sample. In parallel trace Le^b activity could also be detected in the glycolipids of the Le(a+b−) sample and could be immunohistologically demonstrated to be fully expressed in occasional cells in the deep glands of the small intestine, a pattern quite dissimilar to that of the Le(a−b−) nonsecretor. The results in this paper show that the expression of Lewis glycoconjugates in the small intestine parallel the expression of Lewis erythrocyte phenotypes. However, inappropriate Lewis activity is also seen in individuals of other phenotypes and the mechanisms by which these Lewis antigens are made appears to be different for different phenotypes.

Keywords: Lewis antigens, glycolipids, Le(a+b+), Le(a−b−) nonsecretor, small intestine

Abbreviations: FITC, fluorescein isothiocyanate; HPLC, high-performance liquid chromatography; NeuAc, *N*-acetyl-D-neuraminic acid; RBC, red blood cell; TLC, thin-layer chromatography; TRITC, tetramethyl rhodamine isothiocyanate.

Introduction

The Lewis system at the red blood cell (RBC) phenotypic level is a relatively simple system comprised of two major antigens Le^a and Le^b. The formation of Lewis antigens involves the genetic products of the Lewis and secretor loci interacting and competing with each other (when genetically appropriate) to form the known Lewis antigens (as reviewed in [1, 2]). As a result of these interactions two main RBC Lewis groups are usually found, being Lewis negative and Lewis positive. Because the Lewis system is genetically independent of the secretor system, Lewis negative and positive individuals can be either secretors or nonsecretors. In Lewis negative individuals the secretor genotype does not alter the Lewis negative phenotype but in Lewis positive individuals the nonsecretor genotype causes the RBCs to phenotype as Le(a+b−) and the secretor genotype causes

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the Le(a−b+) RBC phenotype. In individuals usually of Polynesian or Asian descent a further RBC phenotype Le(a+b+) can be found. This phenotype, which is virtually absent in Caucasians, is relatively common in Polynesians and Asians and is believed to be caused by an inefficient secretor α1,2-fucosyl transferase [3]. The expression of Lewis antigens is also more complex than that observed at the RBC phenotyping level because Lewis antigens can be seen in Lewis negative individuals [4–6].

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 [1], and as glycolipids are acquired by cells of the peripheral circulation [7–9]. The actual site of synthesis of the plasma Lewis glycolipids is uncertain but it has been suggested that they may originate from the intestinal tract [10]. This is based on the observation that human small intestinal mucosal scrapings and epithelial cells contain large amounts of blood group ABH and Lewis glycolipids [11–13] under the control of the secretor and Lewis genes [13].

The present work describes the immunochemistry and immunohistology of Lewis glycoconjugate expression in the small intestine of two individuals of uncommon phenotypes (Le(a+b+) and Le(a−b−) salivary ABH nonsecretors) and those of common Lewis phenotypes.

Experimental

Samples

Human small intestine (jejunum and ileum) was obtained from cadavers within 36 h post mortem. Most samples were of Polynesian descent because this race was being specifically targeted to find the Le(a+b+) phenotype which is known to be frequent in these people [14]. The intestine was dissected longitudinally and a sample of jejunum was taken for histology at this stage and fixed in 2% formol saline. The dissected intestine was then gently washed free of particulate matter under running tap water. The intestinal lumen was then scraped with a spoon as described [15] and the resulting tan coloured epithelial cells and mucus obtained were then stored frozen until the glycolipids could be extracted.

A description of the blood group, age, sex, probable cause of death, time delay between death and collection of sample, milligrams of total nonacid glycolipids isolated and the race of the cadaver are shown in Table 1. The racial group was as determined by the police from their routine enquiries.

Lewis phenotypes

The Lewis blood group of the cadavers was determined from washed red cells using commercial polyclonal goat antisera (Ortho Diagnostic Systems Inc., Raritan, NJ).

Antibodies for glycolipid analysis and immunofluorescence

Monoclonal antibodies used in the thin layer chromatography overlay techniques were: anti-Le^a 069 (clone BRIC 87) from D. Anstee, 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 R. Fraser, 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 [6, 16, 17]. For immunofluorescence additional

Table 1. Sample descriptions and thin-layer chromatography lane positions.

TLC lane	*Sample*	*ABO*	*RBC Lewis phenotype*	*Age*	*Sex*	*Probable cause of death*	*Hours since death*	*Glycolipids* (mg)	*Race*
1	529	O	Le(a−b−)	21	M	MVC trauma	12	99	Caucasian
2	536	O	Le(a+b−)	41	M	Brain bleed	20	50	Caucasian
3	076	O	Le(a+b+)	49	F	Heart	24–36	37	Polynesian
4	208	O	Le(a−b+)	43	F	Heart	17	12	Polynesian
5	070	O	Le(a−b+)	43	M	Heart	13	254	Polynesian
6	214	O	Le(a−b+)	45	M	Heart	15	114	Polynesian
7	285	O	Le(a−b+)	44	M	Heart	20	291	½Polynesian
8	409	O	Le(a−b+)	31	M	Head injury	24	78	Polynesian
9	578	O	Le(a−b+)	27	M	MVC trauma	8	33	Caucasian
10	118	A1	Le(a−b+)	48	M	Bronchial asthma	30	114	Polynesian
11	153	A1	Le(a−b+)	35	F	Heart	16	113	Polynesian
12	408	A1	Le(a−b+)	22	M	Heart	6	77	Polynesian
N/A	363	A1	Le(a−b−)	15	M	Heart virus	10	None	Polynesian

MVC, motor vehicle crash.
N/A, glycolipids not available for TLC analysis.

antibodies were used which included anti-Le^{ac} (clone 32IEGE) from J. Galton, Tissue Typing Laboratory UCLA, Los Angeles, CA, USA and affinity purified polyclonal rabbit anti-Le^c (lacto-*N*-biose) and anti-Le^d (H type 1) antibodies. These affinity purified antibodies to the Lewis precursors were prepared by hyper-immunizing rabbits with boiled saliva from a Le(a−b−) nonsecretor and a Le(a−b−) secretor individual respectively [18]. Specific antibodies in the hyper-immune serum were then affinity purified on Synsorb™ synthetic Le^c (Galβ1-3GlcNAc-R) and Le^d (Fucα1-2Galβ1-3GlcNAc-R) saccharides (Chembiomed, Alberta Research Council, Edmonton, Canada). The immunological properties of these antisera have been described elsewhere [6, 19]. The nomenclatures used for antibodies (Le^a, Le^b, Le^c, Le^d) are as accepted by the ISBT [20, 21] and reported elsewhere [16] and while the definition of the Le^c epitope may be debated [22], recent evidence suggests that lacto-*N*-biose can be considered as an Le^c epitope [unpublished].

Glycolipids

Total nonacid glycolipids were isolated as described [23]. Glycolipids were not obtained from the A1 Le(a−b−) secretor (sample 363).

The total glycolipid preparations from the Le(a+b+) and Le(a−b+) (samples 076 and 070 respectively) samples were additionally fractionated by high-performance-liquid-chromatography (HPLC) (LKB, Bromma, Sweden) on a 10 µm silica column (Maxsil 10 silica, model OOH-0060-PO, Phenomenex, CA, USA) using a chloroform:methanol:water gradient (80:20:1 to 40:40:12 by volume). Fractions were dried, accurately weighed, and based on their chromatographic mobility and immunostaining properties, the weights for glycolipids with 5, 6 and >6 sugar residues were then determined.

Immunofluorescence

The paraformaldehyde fixed tissues were paraffin embedded and processed for indirect immunofluorescence after deparaffination. Immunofluorescence was by the method previously described [24].

Thin-layer chromatography and immunostaining

Approximately 6 µg of total nonacid glycolipids were loaded per lane on to high-performance silica gel thin-layer plates (Whatman Ltd, England). Plates were chromatographed in a solvent system of chloroform:methanol:water, ratio 60:35:8 by volume, dried and rechromatographed in fresh solvent. Chemical detection was done with the anisaldehyde reagent [23] and immunostaining was performed by the modified method of Magnani *et al.* [25, 26]. Each plate shown represents a single experiment which was photographed, cut and reordered as listed in Table 1.

Glycolipid epitopes and the approximate number of sugars were identified on the basis of their reactivity with defined reagents and known chromatographic mobilities. The nomenclature used for the blood group active glycolipids is as previously described [6].

Results and discussion

The epithelium of the gastrointestinal tract expresses mainly type 1 chain glycolipid structures under the control of the *Se* and *Le* genes [13] and in human small intestine the epithelial cells express type 1 chain ABH and Lewis glycolipid antigens in concordance with the blood group phenotype of the RBCs of the donor [13]. It is believed that the intestinal tract is the source of the plasma Lewis glycolipids which are secondarily absorbed on to RBCs [10]. This is based on the observation that human small intestinal mucosal scrapings and the epithelial cells contain large amounts of blood group ABH and Lewis glycolipids [11–13]. In general immunochemical staining of glycolipids are very similar between Lewis positive plasmas and intestinal fractions, suggesting that similar glycosyltransferase mechanisms are responsible for the epitopes formed [6, 27]. However, there are different Lewis glycolipid ceramide patterns in plasma and intestine, which suggests that either the intestinal tract is not the only origin of plasma glycolipids, or the transport mechanism, from intestine to plasma, discriminates between ceramide species [6].

In this study total nonacid glycolipid extracts of jejunum and ileum and histological samples of jejunum were immunologically compared and contrasted according to their serological RBC Lewis phenotype. It was found that the expression of glycolipid Lewis antigens in the small intestine in many respects parallels that of plasma and RBCs as previously reported [13]. The results here are consistent with accepted biosynthetic pathways for the formation of Lewis a and b antigens, where the Se fucosyltransferase usually competes more effectively for precursor than the Le fucosyltransferase [1].

Intestinal glycolipids

Glycolipids were separated by TLC and stained with the chemical reagent anisaldehyde which detects lipids and gives a characteristic green colour for glycolipids [23] (results not shown). As expected very little di- and triglycosylceramides were present in these glycolipid extracts [13], however lactotetraosylceramide, H-5-1, Le^a-5, and Le^b-6 could be seen as the dominating glycolipids in accordance with blood group phenotypes.

Anti-Lewis antibody glycolipid staining of total glycolipids

Antiserum 069 anti-Le^a reacts with the Le^a epitope, cross-reacts with the type 1 precursors, but does not react with the Le^b epitope [16]. This antiserum (Fig. 1, plate I) reacted strongly with Le^a-5 glycolipids and extended structures of the Le(a+b−) and Le(a+b+) samples (lanes 2 and 3). The pattern of the Le(a+b+) sample was similar to that

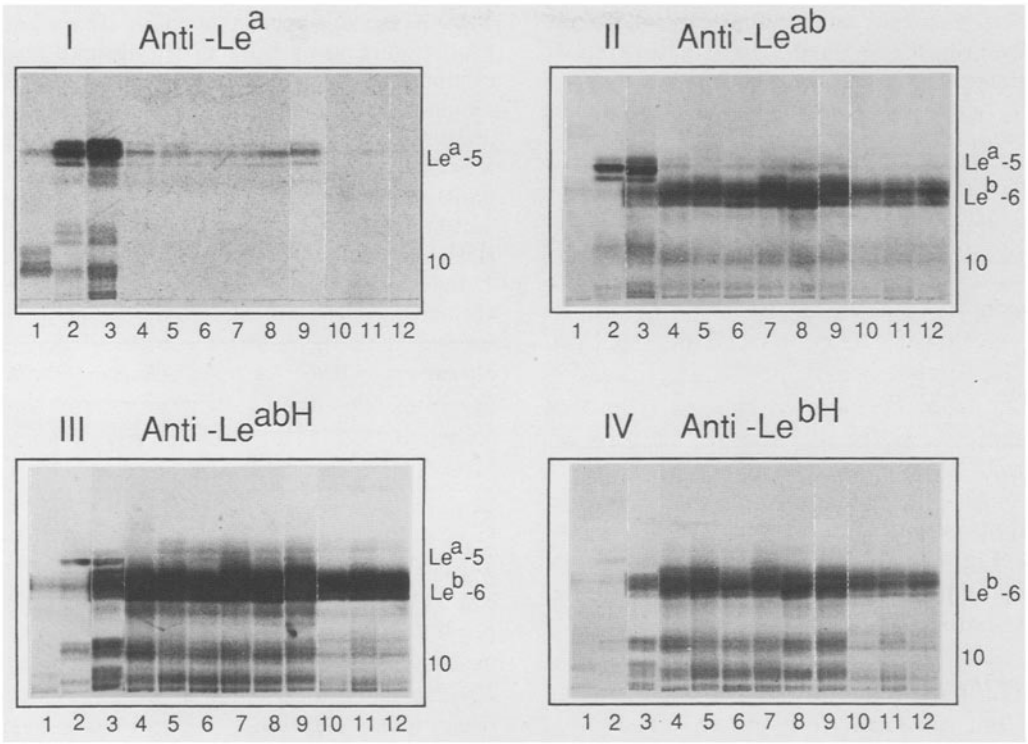

Figure 1. Monoclonal anti-Lewis TLC-CBAs of small intestinal epithelial cell nonacid glycolipids. Lane numbers correspond to sample descriptions in Table 1. Plate I = 069 anti-Le^a, plate II = 073 anti-Le^{ab}, plate III = 074 anti-Le^{abH} and plate IV = 075 anti-Le^{bH}.

of the Le(a+b−) sample, with the exception that reactivity was relatively more intense for the extended structures. This antiserum did not react with the type-1 precursor but did detect Le^a in the Le(a−b−) nonsecretor (lane 1) in both the 5 and 9–12 sugar regions. The binding in the 5 sugar region has subsequently been confirmed by NMR to be Le^a [unpublished].

Antiserum 073 anti-Le^{ab} reacts with both Le^a and Le^b but not the ALe^b epitopes [16]. This antiserum (Fig. 1, plate II) clearly identified Le^a-5 in the Le(a+b−) and Le(a+b+) samples (lanes 2 and 3). Le^b-6 was clearly identified in the Le(a−b+) and Le(a+b+) samples (lanes 3–12). It was clear that the profile of the Le(a+b+) samples was unlike that of other phenotypes with both Le^a-5 and Le^b-6 glycolipids co-expressed in significant quantities. Extended structures with similar migratory profiles were seen for most samples, although this reagent does not stain these substances as strongly as other reagents.

Antiserum 074 anti-Le^{abH} reacts with the Le^b epitope, shows crossreactivity with Le^a and H type 1 epitopes, and does not react with the ALe^b epitope [16]. This antiserum has previously been shown by TLC to react strongly with extended structures in Polynesians [17].

This antiserum (Fig. 1, plate III) reacted strongly with Le^b-6 for all the samples which serologically phenotyped as Le^b positive (lanes 3–12). The Le(a−b−) and Le(a+b−) samples (lanes 1 and 2) also both showed weak reactivity in the Le^b-6 region.

Reactivity was seen with structures with extended chains for all samples. The Le(a−b−) nonsecretor however only showed faint reactivity in the >10 sugar region. All other samples showed similar banding patterns for these extended structures. The observation that these extended structures were much weaker in the group A samples (lanes 10–12) suggests that these extended precursors bear an H type 1 epitope which is utilized by the A transferase before the Le transferase. This results in A and ALe^b epitopes which are unreactive with this antiserum.

Antiserum 075 anti-Le^{bH} reacts with Le^b and related H epitopes but does not react with the Le^a epitope [16]. It is also reported to show weak activity with the ALe^b epitope but does not detect these glycolipids by TLC. This antiserum has previously been shown by TLC not to react with Le^a-5 glycolipids, but to instead react strongly with H-5-1 glycolipids [17]. This antiserum (Fig. 1, plate IV) reacted strongly with Le^b-6 in all the Le^b reactive samples (lanes 3–12). Slight reactivity was again seen in the 6 sugar region for the Le(a−b−) and Le(a+b−) samples, consistent with weak Le^b-6 expression. H-5-1 was not seen other than in trace amounts in some samples.

Reactivity of extended substances in the 9–12 and greater sugar regions are seen. Once again the decrease in intensity of these bands in the group A samples is consistent with the consumption of the extended H type 1 precursors by the A transferase. The reactivity seen may be due to extended Le^b alone or extended Le^b and H together.

Table 2. Approximate glycolipid contribution by dry weight, relative to sugar size in Le(a−b+) and Le(a+b+) individuals. Weights were determined after HPLC fractionation and chemical and immunostaining to identify sizes. Only glycolipids with more than 4 sugars are indicated.

	Glycolipid size			
Sample	*5 sugars*	*6 sugars*	*>6 sugars*	*Total >4 sugars*
070	3.9 mg	41.5 mg	5.6 mg	51.0 mg
Le(a−b+)	8%	81%	11%	100%
076	9.0 mg	3.6 mg	3.9 mg	16.5 mg
Le(a+b+)	54%	22%	24%	100%

Because the banding pattern seen is similar to that of the anti-Le^{ab} reagent (Fig. 1, plate II), a reagent that does not react with H type 1, this suggests that an extended Le^b epitope is being visualized.

HPLC fractions of the Le(a+b+) and a Le(a−b+) sample

The total nonacid glycolipids from the Le(a+b+) and a Le(a−b+) sample were fractionated by HPLC. Based on chromatographic mobility, immunostaining properties, and dry weight of each HPLC fraction, the total weight and percentage composition for glycolipids with 5, 6 and >6 sugar residues were determined (Table 2). It is important to note that the figures in this Table represent only single individuals for each phenotype and it would be expected that variation on these values will be found in other individuals of the same phenotype. All the same, by weight, it is clear that the Le(a−b+) individual made about 10 times more 6 sugar than 5 sugar glycolipids, while the Le(a+b+) individual made about 2.5 times more 5 sugar than 6 sugar glycolipids. Although the exact identity of the 5 and 6 sugar glycolipids is unknown, it would be expected that they would be predominantly Le^a-5 and Le^b-6 [13]. Of the glycolipids with more than 4 sugars, by weight, the amount of extended glycolipids found in the Le(a+b+) individual was about a quarter, while it represented only just over a tenth in the Le(a−b+) individual.

Immunohistology of the small intestine

Tissue sections from the cadavers from whom glycolipids had been extracted were immunohistochemically analysed. Additionally a sample from a group A1 Le(a−b−) presumed secretor cadaver (sample 363) was analysed. Post mortem degradation of samples was evident with the most severe degradation occurring in the group A samples. With the exception of the group A samples, the brush border of epithelial cells had the strongest positive reactions with all the anti-Lewis reagents (Fig. 2, plate A), and could be easily interpreted. The brush border was the best structure for comparison of RBC and intestinal Lewis phenotypes (Table 3). However, for all samples the deeper areas of the crypts were usually better preserved and goblet cells, epithelial cells and deep glands could be clearly identified.

Table 3. Immunostaining of group O cadaver small intestine brush border. Samples have been clustered according to erythrocyte phenotypes and immunohistological results. The Le(a−b−) sample 529 is presumed to be from a nonsecretor. Antisera used are: anti-Le^c, affinity purified rabbit polyclonal anti-lacto-*N*-biose; anti-Le^{ac}, 32IEGE monoclonal anti-Le^a crossreactive with Le^c; anti-Le^{ab}, 073 monoclonal anti-Le^b crossreactive with Le^a; anti-Le^{bH}, 075 monoclonal anti-Le^b crossreactive with H type 1 (Le^d) and anti-Le^d, affinity purified rabbit polyclonal anti-H type 1. Immunohistological results are graded from negative (−) to very strong (+++).

Sample	*RBC phenotype*		*Reactivity with antibodies*				
	ABO	*Lewis*	*Le^c*	*Le^{ac}*	*Le^{ab}*	*Le^{bH}*	*Le^d*
529	O	a−b−	+++	+++	−	−	+/−
536	O	a+b−	+++	+++	+++	−	−
076	O	a+b+	+	++	+	++	−
214	O	a−b+	−	+++	++	+++	+++
208	O	a−b+	−	++	+	++	++
285	O	a−b+	−	+	++	+++	+++
409	O	a−b+	−	+	++	+++	+++
070	O	a−b+	−	+/−	++	++	+++
578	O	a−b+	−	−	++	+++	+++

It was found that the group O Le(a−b−) nonsecretor individual expressed large amounts of Le^c and no Le^a or Le^b at the brush border. A trace amount of activity was detected with affinity purified anti-Le^d but was not confirmed by the anti-Le^{bH} antiserum 075 which crossreacts with Le^d. This reactivity therefore is probably not due to Le^d expression at the brush border. These observations were as expected and correlated well with the RBC phenotype. We were able to show that in the Le(a−b−) nonsecretor, Le^b is expressed on the Golgi apparatus of the deep areas of the Lieberkühn's crypts (Fig. 2, plate B). In this plate reactivity is demonstrated with anti-Le^b 074 but this reactivity was also demonstrated with four of five other anti-Le^b reagents including 075 but excluding 073 (results not shown). In both A and O Le(a−b−) tissue donors, occasional isolated glands with all cells positive for anti-Le^b were found (Fig. 2, plate F).

Ørntoff and co-workers have also demonstrated Le^a and Le^b antigens and detected low α1-4 fucosyltransferase activity in genuine Le(a−b−) individuals (that is, those lacking saliva Lewis transferase activity) [28]. They immunochemically and immunohistologically show Lewis antigens to be present in healthy colonic tissue but did not detect Lewis antigens in the serum of the Lewis negative

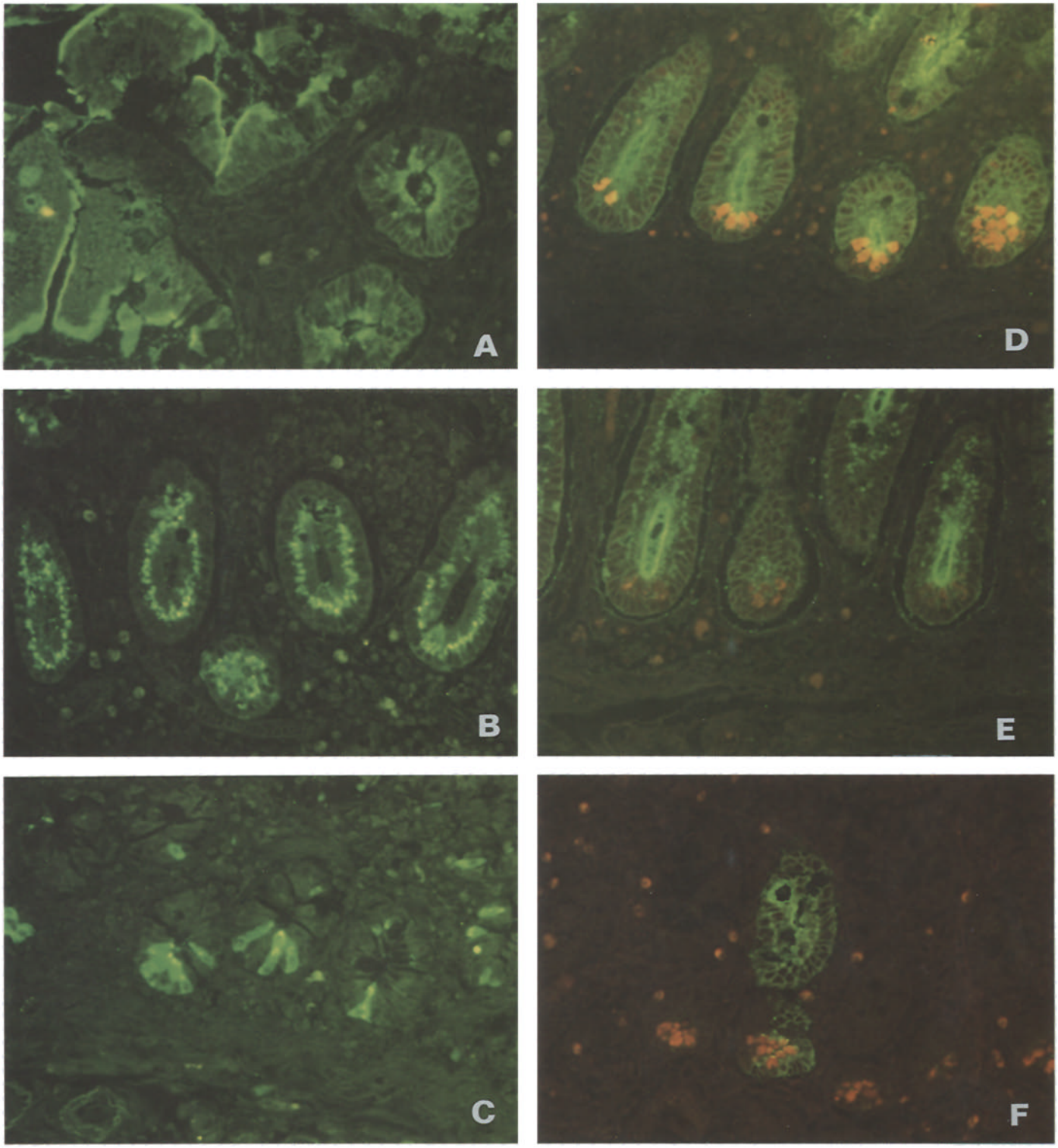

Figure 2. Caption over page.

individual studied. A more recent study has however been able to show the presence of Lewis antigens in the plasma of Lewis negative individuals [6]. Additionally Lewis antigens have been found to be expressed in epithelial cells and nonkeratinized epithelium of labial mucosa in Lewis negative individuals [5].

The Le(a+b−) sample expressed large amounts of Le^a and no Le^b at the brush border (Table 3). Large amounts

◀ **Figure 2.** Expression of Lewis antigens on small intestinal mucosa. Normal pattern of expression on surface villi (plate A) and Lieberkühn's glands (plates D and E) of Lewis antigens corresponding to the red cell Lewis phenotypes of the tissue donors. Three examples of aberrant expression of Lewis antigens, unexpected from the red cell phenotype, are also shown: Golgi pattern with anti-Le^b, on a red cell O Le(a−b−) individual (plate B); staining with anti-Le^{bH} of isolated cells in otherwise negative glands of a red cell Le(a+b−) individual (plate C); and staining of all cells from a short segment of a single isolated gland from a red cell Le(a−b−) individual with anti-Le^{bH} (plate F). Plates D, E and F are double stained with FITC anti-Lewis reagents and TRITC labelled anti-NeuAc lectins, showing in addition to the Lewis staining (green), the staining of cytoplasmic granules of Paneth cells and mast cells (red).

Plate A. Surface villi of a red cell blood group O Le(a−b−) nonsecretor individual (sample 529), stained with the affinity purified rabbit anti-Le^c antibodies. Strong positive reaction of brush border and moderate reactions on basolateral membranes, Golgi, and cytoplasm of all epithelial cells. The same staining pattern was observed with anti-Le^d in the Le(a−b−) salivary secretor, with anti-Le^a in the Le(a+b−), and with anti-Le^b in Le(a−b+) individuals (not shown).

Plate B. Aberrant Golgi Le^b staining pattern. Lieberkühn crypts of the same O Le(a−b−) nonsecretor individual (sample 529), stained with anti-Le^{abH} (sample 074). Brush border and epithelial cells in general were negative, with the exception of strong supranuclear fluorescent spots in the area corresponding to the Golgi apparatus. This Golgi pattern was not seen in the group A1 Le(a−b−) secretor individual (see plate F).

Plate C. Aberrant Le^b staining pattern. Lieberkühn crypts of a red cell O Le(a+b−) individual (sample 536), stained with the anti-Le^{bH} monoclonal antibody (075). Only a few isolated cells were positive in deep areas of some glands with this antibody. The brush border and all epithelial cells of this tissue samples were strongly positive with the anti-Le^a antibodies (not shown).

Plate D. Lieberkühn crypts of the O Le(a−b−) nonsecretor individual (sample 529) double stained with anti-Le^c (green) and TRITC labelled *Maackia amurensis* lectin (red). Strong staining of all the epithelial cells, with the expected anti-Lewis reagent (green) corresponding to the red cell and secretor phenotype of the tissue donor, as in plate A. Only the Paneth cells show strong granular fluorescence in the cytoplasm with the anti-NeuAc lectin (red).

Plate E. Lieberkühn crypts of a blood group O Le(a−b+), salivary secretor individual (sample 578) double stained with affinity purified rabbit anti-Le^d antibodies (green) and the TRITC labelled *Sambucus nigra* lectin (red). The anti-Le^d antibody stained cell membranes, Golgi and cytoplasm of all epithelial cells (green), in the same way as the anti-Le^b antibodies (not shown), but only cytoplasmic granular staining of Paneth cells was seen with the anti-NeuAc lectin (red).

Plate F. Aberrant Le^b staining of a single portion of an isolated gland. Lieberkühn crypts of a blood group A1 Le(a−b−) secretor individual (sample 363) double stained with anti-Le^{bH} (075) (green) and the TRITC labelled *Maackia amurensis* lectin (red). All the mucosa including surface (not shown) and deep areas were negative with anti-Le^{bH} in the 6 cm strip of this tissue sample, with the only exception of a single isolated gland which showed a short segment where all epithelial cells were positive (green). The cytoplasmic granules of Paneth cells had the same strong fluorescence with the anti-NeuAc lectin (red) as seen in plates D and E. In addition, cytoplasmic granules of isolated mast cells throughout the tissue were also seen positive (red).

of unconverted Le^c were also detected and Le^d was absent in the brush border. Le^b reactivity was also found to be expressed in 10–20% of isolated cells, mainly located in the deep areas of the Lieberkühn's crypts (Fig. 2, plate C). In this plate reactivity is demonstrated with anti-Le^{bH} 075 but this reactivity was also demonstrated with two or five other anti-Le^b reagents (results not shown). This mosaic pattern has also been reported for acinar cells of a Le(a−b−) secretor stained with anti-Le^b [5]. It is of interest to note that this mosaic pattern of reactivity is unlike that of the Le(a−b−) nonsecretor where Le^b reactivity is expressed only in the Golgi of all cells in this region. Mandel also reported inappropriate expression of Le^b in mucosal acinar and spinous tissues of an Le(a+b−) sample [5]. Single isolated positive glands were occasionally found with anti-Le^b.

The Le(a+b+) sample, unlike those of other Lewis phenotypes, co-expressed similar amounts of Le^a and Le^b at the brush border (Table 3). Unconverted Le^c precursor could also be found in the brush border of this Le(a+b+) sample. Of particular note is the nonexpression of Le^d at the brush border of the Le(a+b+) sample, which is clearly present in all the Le(a−b+) samples. In this respect the Le(a+b+) sample resembles the Le(a+b−) sample which also does not express Le^d at the brush border. From this perspective the Le(a+b+) phenotype presents a unique Lewis glycoconjugate profile to the lumen of the intestine tract, where Le^b but not Le^d is expressed. These observations show that the Le(a+b+) phenotype is not only expressed on RBCs and in plasma but is also found in tissue, and represents a distinct glycoconjugate profile.

All the Le(a−b+) samples expressed large amounts of Le^b and variable amounts or no Le^a at the brush border (Table 3). Unconverted Le^c was not detectable but unconverted Le^d precursor activity could be found in the brush border of these Le(a−b+) samples.

In conclusion, the present study, with due precautions, provides evidence that although the small intestine may not be the only source of plasma Lewis antigens, the expression of Lewis antigens in this organ closely parallels the plasma and RBC Lewis phenotypes. We show that the Le(a+b+) phenotype is expressed at the tissue level and presents a unique glycoconjugate profile. We also show that increased chain elongation occurs in this phenotype, a result which is consistent with the postulated action of a weak secretor transferase. Although the chemical structures of these

extended glycolipids have, as yet, not been fully resolved, preliminary results suggest that some of them are similar to the repetitive type 1 chain structures recently described [29, 30]. Inappropriate Lewis antigen expression was found and these aberrant results show that regulation of phenotypic expression is different in different tissues and fluids. The Lewis enzyme is also the only $\alpha(1,3)$fucosyltransferase known to also use type 1 precursor chains as acceptors [31] and it is encoded by the *FUT3* gene [32]. The enzymes encoded by *FUT4* [33], *FUT5* [34] and *FUT6* [35, 36] genes cannot use type 1 acceptors to make Le^a or Le^b antigens. However, the biosynthesis of Lewis antigens may be carried out by fucosyltransferases encoded by new genes, different from the known *FUT3* gene. Another possibility is that the secretor and Lewis negative alleles (*le* and *se*) may give rise to partially active transferases in some individuals. This is supported by the recent findings that the Lewis alleles in some RBC Lewis negative individuals code for a partially active transferase [37].

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