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ORIGINAL ARTICLE

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Expression of type-2 histo-blood group carbohydrate antigens (Le^x, Le^y, and H) in normal and malignant human endometrium

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Abstract Changes in expression of histo-blood group ABH and Lewis antigens are common alterations in carcinomas. Using immunohistochemistry, we have evaluated the expression of type-2 histo-blood group antigens in normal and malignant endometrial tissues in relation to genetic and hormonal factors. The Le^x, sialosyl-Le^x, and Le^y antigens were inconstantly expressed in the normal endometrium. The expression was uninfluenced by the secretor status but was related to the ABO blood group status in Oestradiol (E_2) stimulated endometria. Le^y was expressed most frequently in proliferating endometria from blood group 0 individuals. Le^x and Le^y were maximally expressed in atrophic endometria, and Le^x and Le^y staining scores correlated inversely with serum levels of E_2 in normal, non-secretory endometria. No correlation was found in adenomatous hyperplasias and endometrial carcinomas, which when compared with atrophic endometria, showed a loss of Le^x and Le^y and an increased H-carbohydrate expression at apical membranes. Carcinomas from non-secretors showed lower expression of Le^y and H-antigens than carcinomas from secretors. Our findings suggest that the genetic and hormonal influence on glycosylation based on type-2 chain carbohydrates differ between normal and malignant endometrium. This difference is probably related to specific tumour-associated qualitative and quantitative changes in the fucosyltransferases.

Key words ABO and Lewis blood group antigens
Carbohydrates · Human endometrium
Endometrial carcinomas

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Introduction

Most carcinoma cells show changes in the expression of carbohydrates belonging to the histo-blood group ABH and Lewis antigen systems (Hakomori 1984; Lloyd 1987). An increased fucosylation in addition to deletion

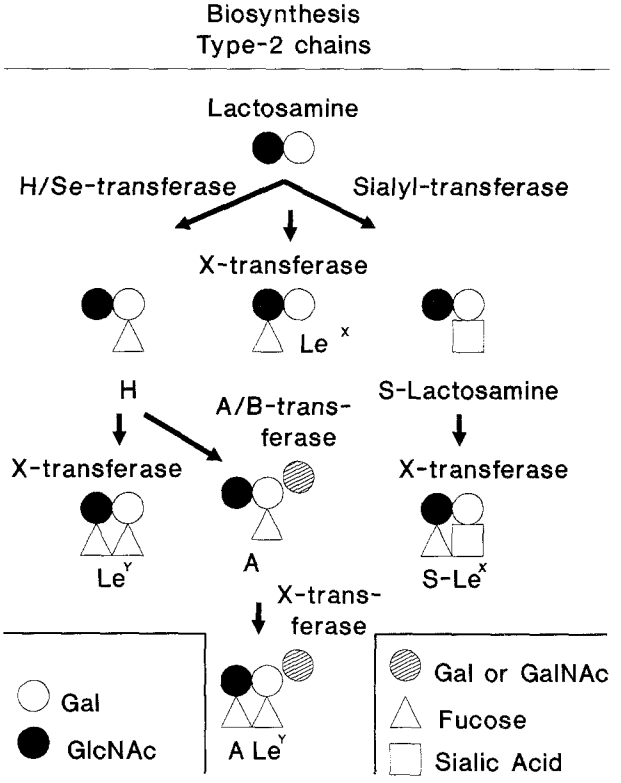


Fig. 1 Structure and proposed biosynthetic pathway for biosynthesis of type-2 chain fucosylated histo-blood group antigens in normal human endometrium. The disaccharide lactosamine can be extended either by action of the X-gene defined or related transferases to the Le^x structure, by the action of the H/Se gene defined fucosyltransferases to the H-antigen, or by sialosyl-transferases to sialosyl-lactosamine. Substrate competition between transferases (e.g. between X-transferase and A/B transferases) for the same substrate (e.g. H-antigen) may influence carbohydrate expression

of A and B antigens seem to explain the accumulation of H, Le^y and Le^b antigens found in many carcinomas (Stellner et al. 1973; Macher et al. 1991; Dennis 1992).

In normal tissues the expression of histo-blood group antigens varies with tissue-type and is influenced by the genetic status of the individual in terms of blood-group ABO, Lewis and secretor status (Clausen and Hakomori 1989) (Fig. 1). Apart from this genetic influence, a hormonal influence on glycosylation has recently been suggested in human endometrial tissue (Thor et al. 1987; Neunteufel and Breitenecker 1989; Aplin 1991; Ravn et al. 1992a, b, 1993). Few studies have dealt with alterations in expression of histo-blood group related antigens in endometrial carcinomas (Inoue et al. 1987; 1988, 1990; Tsuji et al. 1987; Iwamori et al. 1989; Neunteufel and Breitenecker 1989; Menom et al. 1990; Scharl et al. 1991; Shiozawa et al. 1991) and these suggest that the H and the related difucosylated Le^b and Le^y antigens accumulate in endometrial carcinoma cells. The possibility that the expression of these carbohydrates may be related to the genetic and hormonal status has not been considered in previous studies.

In the present study we have evaluated the expression of type-2 chain histo-blood group antigens, the H (MoAb Be2), Le^y (MoAb AH6) and Le^x (MoAb SH1) antigens in different normal, premalignant, and malignant endometrial tissues using immunohistochemistry and specific monoclonal antibodies (MoAbs).

Materials and methods

Biopsies of normal and malignant endometrium were sampled from hysterectomy specimens with informed consent. The tissues were fixed in formalin (~24 h) and embedded in paraffin by routine procedures. Haematoxylin and eosin stained sections were used for the histological diagnosis which was based on conventional criteria (Dallenbach-Hellweg 1987). All available blocks of tissue were investigated if carcinoma or adenomatous hyperplasia was suspected. Endometrial carcinomas were typed, graded, and staged in accord with the recommendations of WHO and FIGO (Poulsen et al. 1975), (Table 2). The biopsies investigated in the present study were included by using the criteria of an optimal tissue preservation, a defined histology (Table 1), and an established erythrocyte ABO and Lewis blood group status (Ravn et al. 1992a). Normal cycling endometria were investigated in a separate study and were not included (Ravn et al. 1992b). Biopsies displaying endometritis or curretage sequelae were excluded.

Details on menstrual history, data of last menstruation, hormonal therapy and previous gynecological surgery were obtained from questionnaires or clinical records (Table 1).

ABO and Lewis blood typings were performed on erythrocytes, and ABH-saliva secretor status was determined on saliva-samples as described in detail previously (Ravn et al. 1992a). If no salivary secretor status was available, the erythrocyte Lewis phenotype was used to predict the secretor status in Le^{a-b+} (secretors)

Table 1 A The material (*No. Inv.* number of specimens investigated; *Hormone Ana* number of women on which a hormone analysis was available; *Hormone Treatm.* number of women in hormonal substitution therapy at the time of h ysterectomy or within one month prior to hysterectomy; * the number of women who for more than 1 month ago received hormonal treatment is given in the brackets. Data on the hormonal therapy given at the time of operation is given in Table 1B; †tested on saliva; *a* saliva-secretor, *b* saliva status determined on 7 of 12 Le^{a-b-} individuals; *Atrophic* includes inactive endometria; *Weak Prol* weak or insufficient proliferation; *Norm Prol* normal proliferative; *Irreg Prol* irregular proliferative; *AH* adenomatous hyperplasia, all grades included; *EC* endometrial carcinomas, graded and typed by the recommendations of WHO (Poulsen et al. 1975) and FIGO)

Morphology	No. Inv	Blood group ABO and Lewis status					Saliva Status†	Hormone		Age Median (range)
		0		A/B		ABO		Ana	Treatm*	
		La^{a-b+}	Le^{a+b-}	Le^{a-b+}	Le^{a+b-}	Le^{a-b-}				
Atrophic	17	6	1	9	1		15	12	2 (5)	63 (46–76)
Weak Prol.	10	3	0	5	2		8	6	0 (6)	50 (31–52)
Norm Prol.	7	2	0	5	0		4	2	1 (3)	47 (40–50)
Irreg Prol.	9	3	0	4	2		9	5	2 (4)	49 (43–63)
AH	13	1	2	6	3	1[a]	7	7	2 (6)	63 (51–67)
EC Gr. I	33	12	2	11	3	5[b]	16	13	11 (10)	66 (47–84)
EC Gr. II	17	5	1	6	1	4[b]	8	6	2 (8)	65 (58–88)
EC Gr. III	6	1		1	2	2[b]	2	3	1 (1)	65 (53–71)
Total	112	33	6	47	15	12	69	54	21 (43)	(31–88)

Table 1B The hormonal treatment regimen for women receiving hormonal treatment at the time of hysterectomy or within one month prior to operation (E_2 oestradiol, human natural was given as tablets or by injections; *P* progestagens were given in the second half of the cycle, or continuously[a])

Morphology	No. inv/total	Pure E_2	Pure P	Sequential E_2+P	Combined E_2+P
Normal	5/43	2	1	1	1
Adenomatous hyperplasia	2/13	0	0	2	0
Endometrial carcinomas	14/56	5	1[a]	8	0
Total	21/112	7	2	11	1

Table 2 Histological grade and FIGO stage of carcinomas investigated

[a] One adenosquamous and one endometrioid carcinoma
[b] One case had a clear cell carcinoma coponent
[c] All cases listed included serous carcinoma element, in one case as the only component. Remaining 50 carcinomas were pure endometroid, in 13 cases including secretory or mucinous carcinoma

FIGO stage		Grade 1	Grade 2	Grade 3	All grades
1A	No myometrial invasion	5	1	0	6
1B	<50% Myometrial invasion	21	8	0	29
1C	>50% Myometrial invasion	2	3	2	7
2A	Involvement endocerv. glands	2	0	0	2
2B	Invasion cervical stroma	3	3[b]	0	6
3A	Metastasis to serosa/adnexa	0	1[c]	2[c]	3
3B	Metastasis to vagina	0	1[c]	0	1
4A	Invasion bladder/rectum	0	0	2[a]	2
4B	Distant metastasis	0	0	0	0
All stages		33	17	6	56

Table 3 Specificity of antibodies reacting with type-2 chain mono- and difucosylated carbohydrate antigens (Sialosyl-Le^x was demonstrated using the SH1 MoAb on neuraminidase pretreated sections. The difference in staining in neuraminidase pretreated compared to staining in untreated sections indicate presence of sialosyl-Le^x)

	Antigen determinant	Antibody/Ig-class/Reference
Le^x	Galβ1-4[Fucα1-3]GlcNAcβ1$\rightarrow$R	SH1/IgG3/Fukushi et al. 1984
Chain 2 H	Fucα1-2Galβ1-4GlcNAcβ1$\rightarrow$R	BE2/IgM/Young et al. 1981
Le^y	Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ1$\rightarrow$ R	AH6/IgM/Abe et al. 1983

and Le^{a+b-} (non-secretors) individuals (Watkins 1966; Hartman 1941; Ørntoft et al. 1991).

Blood samples for hormone analysis were drawn the morning before hysterectomy from 54 of the 124 women included for immunohistochemical study (Table 1). The serum concentrations of the following hormones were measured: Oestrone (E_1), 17β-Oestradiol (E_2), Oestrone sulphate (E_1SO_4), sex hormone binding globulin (SHBG), non-protein-bound E_2 (free E_2) and non-SHBG-bound E_2.

E_1 and E_2 were measured by radioimmunoassay after extraction with diethyl ether and chromatography on columns of LH 20 using specific rabbit antibodies raised against 6-keto-E_1 and 6-keto-E_2 coupled to bovine serum albumin via the 6-O-(carboxymethyl)-oximes (Emment et al. 1972). E_1SO_4 was measured by radioimmunoassay as E_1 after further ethyl acetate extraction, solveolysis and column chromatography (Franz et al. 1979). The concentration of SHBG was estimated as the binding capacity of ^{3}H-dihydrotestosterone (Gluud and Bennet 1986). Measurement of the percentage of free E_2 was carried out by the centrifugal-ultracentrifugal-dialysis method (Hammond et al. 1980). The percentage of non-SHBG-bound E_2 was performed by incubating serum with ^{3}H labelled E_2 and precipitation of the ^{3}H E_2-SHBG complex with ammonium sulphate (Tremblay and Dube 1974).

For E_1, E_2, E_1SO_4, SHBG, free E_2 and non-SHBG-bound E_2 inter-assay coefficients of variation were 9.6%, 8.5%, 10.3%, 7.5%, 10.5%, and 6.4%, respectively. The intra-assay coefficients of variation were 7.0%, 7.4%, 7.0%, 5.2%, 8.6%, and 5.2%, respectively. The assay sensitivities were 40 pmol/l for E_1 and E_2, 200 pmol/l for E_1SO_4 and for SHBG 5 nmol/l.

Mouse MoAbs with specificity to chain-2 H (Be2), Le^y (AH6), and Le^x (SH1) were used for immunocytochemistry. Their specificity, source, immunoglobulin subclass and reference for specificity are listed in Table 3. All antibodies were used as undiluted culture supernatants. Neuraminidase type X (Sigma) from *Clostridium perfringens* (0.1 unit pr ml acetate buffer with 0.04 M calcium chloride added) was used to remove sialic acid from sialylated antigens i.e. to demonstrate presence of sialosyl-Le^x (sLe^x) (Dreznick 1973). Peroxidase-conjugated rabbit anti-mouse immunoglobulin (P260, DAKO, Copenhagen), diluted 1: 20 was used as second layer antibody.

After deparafinisation and rehydration some of the serially cut sections were pre-incubated with neuraminidase and all subsequently stained by an indirect two layer immunoperoxidase staining technique using overnight incubations of the first layer antibodies and 0.04% 3-amino-9-ethyl carbazole (AEC) as chromogen as previously described in detail (Ravn et al. 1992a). Only selected case were used.

Staining was controlled by replacing the primary antibody with diluent buffer, a MoAb of irrelevant specificity but with the same isotype, and culture supernatant. In addition sections of cervical tissues with known ABO, Lewis blood group and saliva secretor status served as positive and negative tissue controls (Torrado et al. 1990).

Neuraminidase treatment was controlled by pretreatment with acetate buffer alone, neuraminidase pretreatment abolishing staining with MoAbs to sialylated antigens, for example the sialosyl-Tn (MoAb TKH2), and staining of endothelium by MoAbs to T-antigen (MoAb HH8) and lactosamine (MoAb 1B2). This procedure was adopted exclusively in neuraminidase pretreated sections (Ravn et al. 1992a, b).

The immunohistochemical staining was scored semi-quantitatively in arbitrary staining scores ranging from 0 to 4 as follows: score 0, no stain, score 1, <10% of the cells stained, score 2 between 10 and 25%, score 3 between 25 and 75%, and score 4 >75% of the cells stained. This system was previously shown to be reproducible by Kappa statistics (Ravn et al. 1992a), and by comparing staining in different blocks of normal corpusfundal endometrium from the same hysterectomy specimen (Ravn et al. unpublished observation).

Spearman's rank correlation and the Mann Whitney tests were used to evaluate the correlation between arbitrary staining scores and hormone levels, and differences in arbitrary scores between subgroups, respectively, by using the computer programme Medstat (Astra, Copenhagen). A P value <0.05 was chosen as the level of significance.

Results

The MoAb to H-antigen stained endothelial cells in 86% and 71% of endometria from O and A_2 individuals, respectively, whereas endothelium from A_1 individuals stained less frequently (24%). The MoAb to Le^x/sLe^x stained granulocytes. Stromal cells were unstained.

The expression of the carbohydrate structures investigated varied in epithelial cells. Despite this, distinct pat-

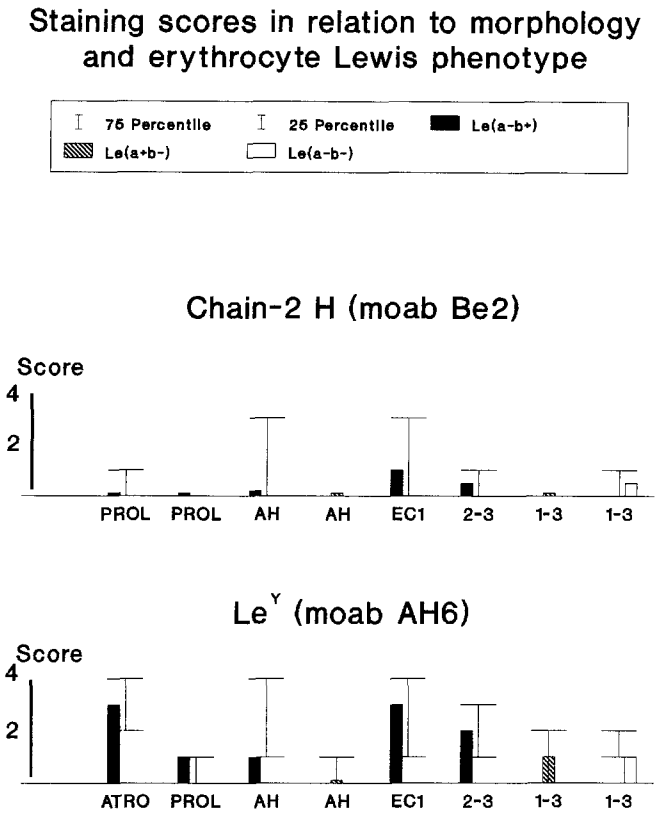


Fig. 2 Median staining scores (boxes) and 25% and 75% percentiles for staining with MoAbs to chain-2 H (Be2) and Le^y (AH6) in relation to morphology and erythrocyte Lewis phenotype. Staining scores include staining of membranes and cytoplasm. *Atro* atrophic; *Prol* proliferative; *AH* adenomatous hyperplasias; *EC* endometrial carcinomas; *1* grade 1; *2* grade 2; *3* grade 3

terns of staining in epithelial cells relating to the genetic background and the morphology were evident in the functionalis glands and are described in detail below and in Figs. 2–4. There was no visible difference in staining in morphologically similar endometria from women on hormonal therapy when compared with staining in women who were not (Table 4). Therefore, the staining results for these subgroups have been pooled.

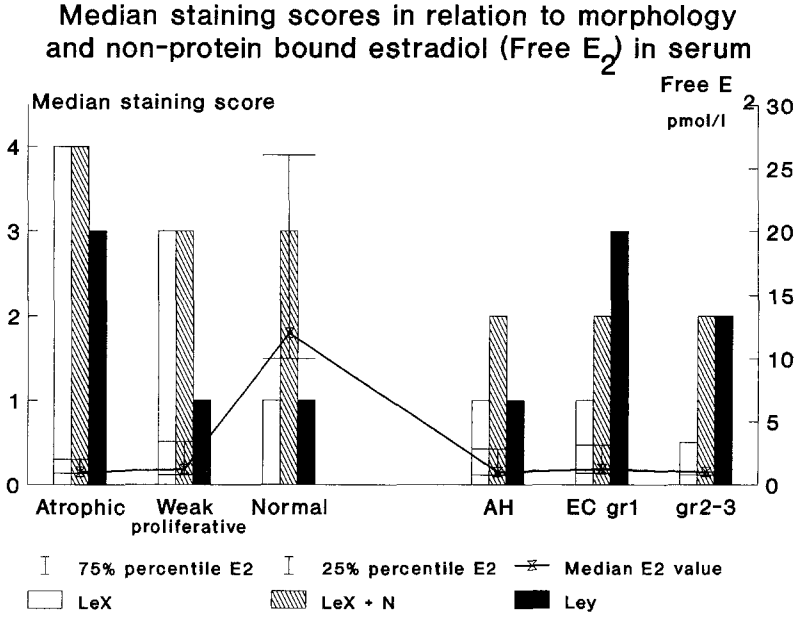
Some variations in staining were related to the layers. In normal endometrium, the Le^x and Le^y antigens showed highest staining scores in the basal layer glands, and Le^x antigen was expressed predominantly in the deepest "invasive" parts of endometrial carcinomas.

In general, normal endometrium expressed type-2 chain carbohydrates at apical membranes predominantly. In neoplastic endometrial cells, however, they were demonstrable at both apical membranes and in the cytoplasm, and all carbohydrates investigated, except for the H antigen, tended to accumulate in the cytoplasm, whereas expression at apical membranes declined (Fig. 4).

The secretor status had no influence on staining for Le^x and Le^y antigens in normal tissues. H-antigen was infrequently expressed (few cells stained in 2/43 normal endometria) and exclusively in endometria from secretors (Fig. 2).

In proliferating endometria (weakly-, normal-, and irregular proliferating endometria), the antibody to Le^y antigen stained more cells in endometria from blood-group O than in endometria from blood-group A individuals (P=0.0013). It was impossible to see any difference in expression of Le^x, and of Le^y in atrophic endometria, in relation to the ABO blood-group status. The H antigen was exclusively demonstrated in normal endometria from blood group O secretors.

Fig. 3 Median staining scores for staining with MoAbs to Le^x (□), Le^x in neuraminidase pretreated sections (Le^x+sLe^x) (▨), and Le^y (■) in relation to morphology and median serum levels of free E_2 (X). The staining scores include staining of membranes and cytoplasm. *Atrophic* includes atrophic and resting endometrium; *Weak* weakly or insufficient proliferation; *Normal* includes normal and irregular proliferative endometria; *AH* adenomatous hyperplasia, all grades; *EC* endometrial carcinomas, grades 1 to 3



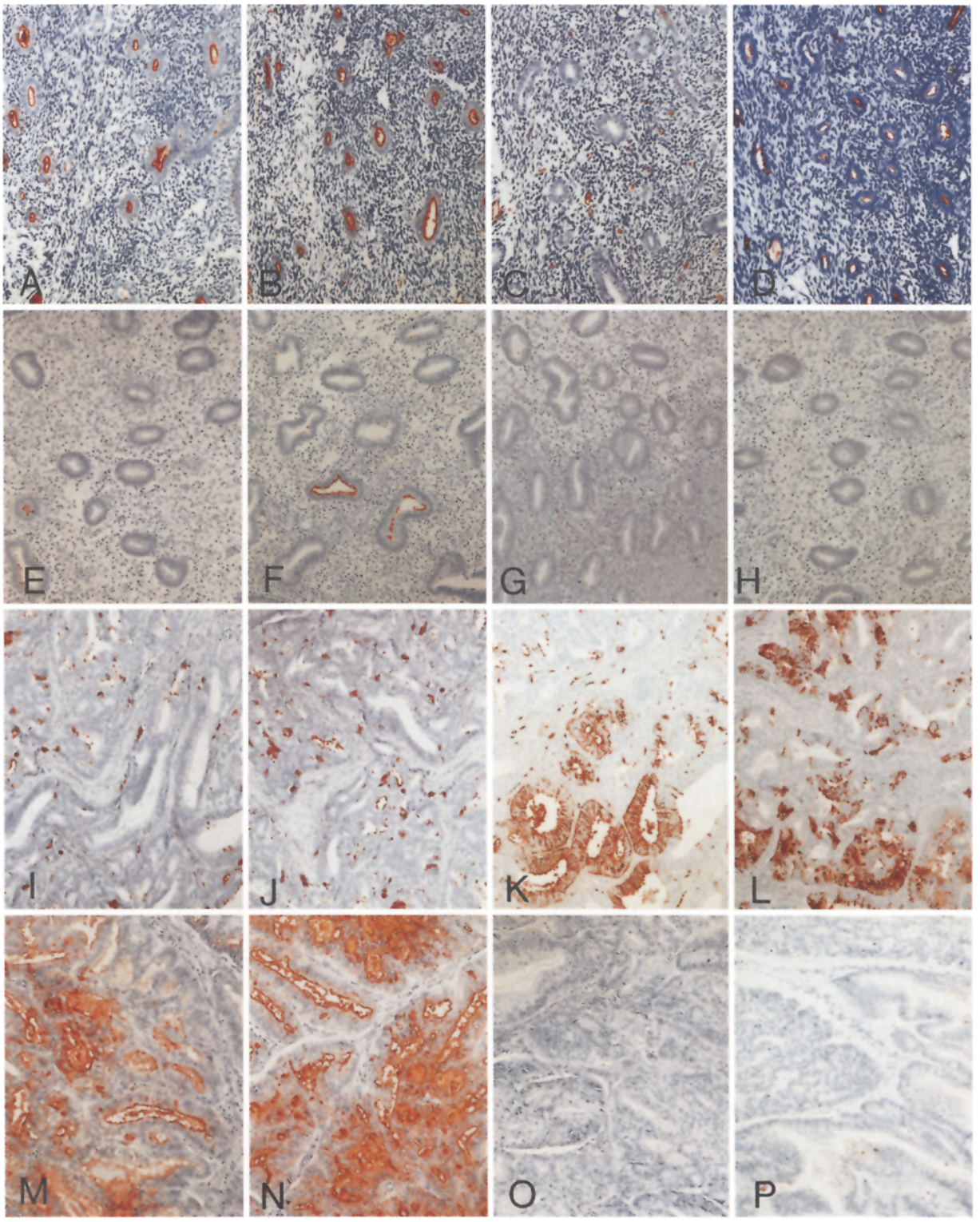


Fig. 4 Immunohistochemical staining for Le^x in neuraminidase untreated (**A, E, I, M**), and in neuraminidase pretreated (**B, F, J, N**) endometria, and with MoAbs to H chain-2 (**C, G, K, O**), and Le^y antigens (**D, H, L, P**) in atrophic (**A–D**), proliferative endometria (**E–H**), a grade 1 endometrial carcinomas from a secretor (**I–L**), and from a non-secretor (**M–P**)

Forty-one of 43 normal endometria stained for Le^x. Normal and irregular proliferative endometria showed a varied staining (scores range 0–3), but, in general, only few cells expressed Le^x (Fig. 4E). In contrast, most cells were consistently stained in atrophic endometria (Figs. 3, 4A).

After neuraminidase treatment all specimens stained

Table 4 Staining in grade 1 endometrial carcinomas from secretors in relation to hormonal treatment group (*Horm.Treat* hormone treatment; *No. Inv.* number investigated)

Antigen	Median staining score and range (in brackets)		
	+Horm.Treat (No. inv.=7)	–Horm.Treat (No.inv.=18)	*P*-values* (two-sided)
Le^x	2 (0–3)	1 (0–3)	0.16151
Le^x+sLe^x	2 (1–4)	2 (1–4)	0.85715
Le^y	2 (1–4)	3 (0–4)	0.67449
H	1 (0–3)	1 (0–4)	1.00000

* The Mann-Whitney (Rank sum) test

for Le^x. In proliferative endometria more cells were stained when compared with staining in untreated sections (Fig. 4F). Atrophic endometria stained as they did before neur- aminidase treatment (Figs. 3, 4B). In addition to staining of apical membranes, a focal, finely granular staining of the cytoplasm was found in neuraminidase pretreated sections, mainly in the most basal glands.

The MoAb to Le^y stained 34 of 43 normal endometria, leaving 9 endometria unstained. Only a few cells were stained in actively proliferating endometria (Fig. 4H). In contrast, most cells were stained for Le^y in atrophic endometria (Figs. 3, 4D).

Apical membranes of few, scattered cells in 2 of 37 normal endometria from secretors stained for H (Fig. 2).

In normal endometria, staining scores for Le^x and Le^y were inversely correlated with serum levels of non-SHBG-bound E_2 (Le^x: R(S)=–0.715, P=0.00006, Le^y: R(S)=–0.547, P=0.0047), free-E_2, E_2, and E_1SO_4, but showed no correlation with oestrone (E_1) or SHBG levels. In addition, the difference in staining scores for Le^x in neuraminidase pretreated compared with untreated sections was positively correlated with E_2 levels (R(S)=0.649, P=0.00025 for non-SHBG-bound E_2) (Fig. 3).

Secretors expressed type-2 H-determinants in 25 of 37 carcinomas and in 2 of 7 adenomatous hyperplasias, whereas this antigen was not demonstrated in corresponding endometria from non-secretors (P=0.0025), (Fig. 2). Non-secretors expressed lower levels of Le^y antigen than secretors (Fig. 2, P=0.0085). The ABO blood-group status had no influence on the expression of Le^y and Le^x antigens in adenomatous hyperplasia and endometrial carcinomas. H carbohydrate antigen was, in contrast to the findings in normal endometrium, found in malignant endometrial cells. Immunohistochemical staining did not reveal any difference in expression in relation to the ABO blood group status.

In neuraminidase untreated sections 10 of 13 cases of adenomatous hyperplasia were stained by the MoAb to Le^x. As in the normal endometrium, the proportion of cells stained varied, but in most cases only few cells expressed Le^x (Fig. 3). After neuraminidase pretreatment, more cells stained than before neuraminidase pretreatment, indicating presence of sLe^x, and all specimens were stained. When compared with staining in normal endometrium, however, fewer cells were stained (Fig. 3).

All adenomatous hyperplasias from secretors expressed Le^y at their apical membranes and in some cases in the cytoplasm too. Usually only a few cells expressed Le^y. In one case, however, the MoAb to Le^y stained most cells (Figs. 2–3).

Single cells were stained with MoAb to H antigen at apical membranes in 2 of 7 adenomatous hyperplasias from secretors.

Le^x was expressed in 75% of the adenocarcinomas. The fraction of stained cells varied considerably from case to case, but typically, few cells were stained. Staining scores tended to fall with increasing grade (Figs. 3, 4I, M). After neuraminidase pretreatment more cells were stained, and all specimens stained for Le^x. When compared with normal atrophic endometrium fewer cells were stained (Figs. 3, 4J, N). The fraction of stained cells showed considerable intra- and inter-individual variation, which was unrelated to tumour grade. However, the difference in staining scores between neuraminidase pretreated and untreated sections (sLe^x) tended to increase with increasing grade (Fig. 3).

All but 2 of 37 carcinomas from secretors and 3 of 10 carcinomas from non-secretors expressed Le^y. The fraction of cells stained for Le^y was lower than in the atrophic endometrium in 12 of 23 grade 1 carcinomas (Figs. 3, 4L, P). Moreover, staining predominated in the cytoplasm in most carcinomas (Fig. 4). Le^y staining decreased with increasing histological dedifferentiation (Fig. 3).

Most (18/23) grade 1 carcinomas and half of the grade 2–3 carcinomas from secretors expressed type-2 H antigen at apical membranes of scattered cells (Figs. 2, 4).

No correlation between staining scores for Le^x, Le^y, and H in carcinomas from secretors and the FIGO stage and no correlation between staining scores and serum hormone levels were demonstrable (data not shown).

Discussion

Changes in glycosylation are seen in several physiological and pathological processes. Carbohydrates are involved in early blastocyst adherence and -attachment to the endometrium (Kimber and Lindenberg 1990; Fenderson et al. 1991) and in tumour cell metastasis (Dennis 1992; Miayke et al. 1992). Cell surface carbohydrates are important in the interactions between cells and between cells and the extracellular matrix (Dennis 1992).

The biosynthesis of carbohydrates with blood-group specificity is catalysed by an ordered and sequential action of several different gene-encoded glycosyltransferases that each catalyse specific steps in the synthesis of the carbohydrate chains (Oriol et al. 1986; Clausen and Hakomori 1989) (Fig. 1). Lack of one of the enzymes or cofactors required for the biosynthesis of the precursor chains and competition between enzymes for the same

substrate may influence antigen synthesis and expression.

In the present study we evaluated the expression of fucosylated type-2 chain histo-blood group antigens in human endometrium. We found a varied expression of Le^x, H and Le^y antigens in the endometrium which was found to be related to both genetic (ABO, Lewis and secretor status) and hormonal factors.

The ABO blood-group status, in agreement with previous findings (Ravn et al. 1992b) influenced the expression of Le^y antigen in normal proliferative, E_2 stimulated endometria. However, the ABO blood-group status had no demonstrable influence on the expression of neither Le^x nor Le^y antigens in atrophic endometria, adenomatous hyperplasias or endometrial carcinomas. The H-determinant may act as precursor substrate in synthesis of both A/B and Le^y antigens (Clausen and Hakomori 1989). The A/B transferases which catalyse synthesis of A/B antigens from H structures is expressed only in A/B individuals, and predominantly in E_2 stimulated endometria (Fig. 1). In this tissue the A/B-transferases may compete with the α1-3/4 fucosyl-transferases (the X-transferase needed for Le^y synthesis) for their common substrate, the H-determinant. In atrophic endometria, adenomatous hyperplasias, and endometrial carcinomas only low levels of A/B transferases are generally present and Le^y is therefore synthesized.

As in most other epithelia, the Le^y and Le^x antigens were found to be expressed in a manner uninfluenced by the secretor status in the normal endometrium (Clausen and Hakomori 1989; Ravn et al. 1992b). In adenomatous hyperplasias and endometrial carcinomas, however, expression of both type-2 H and Le^y antigens were found to be related to the secretor phenotype; H- and Le^y antigens had a lower expression in carcinomas from non-secretors than in carcinomas from secretors. Both H and Se genes, which are closely linked on the long arm of chromosome 19 (Ball et al. 1991) code for a distinct α-2-fucosyltransferase which works preferentially but not exclusively on types-2 and −1 precursor chains, respectively (Le Pendu et al. 1985; Oriol 1990; Oriol et al. 1992). Our findings suggest that the H-gene defined α1-2-fucosyltransferase regulate expression of H and Le^y antigens in the normal endometrium. The Se gene-defined α1-2-fucosyltransferase, which convey the secretor dependency, seem rather, in contrast with the findings in normal endometrium, to regulate expression of H and Le^y antigens in carcinoma cells.

Besides genetic factors, our findings suggest that hormonal factors may have an influence on synthesis and expression of type-2 chain H, Le^x, and Le^y carbohydrates in the normal endometrium (Ravn et al. 1992b). The varied expression, shown by staining scores, for both Le^y and Le^x antigens were shown to be inversely correlated to serum-E_2 levels in the present study. In contrast, expression of sialosyl-Le^x antigen showed a positive correlation with E_2 levels. The physiological significance of this finding is obscure. Meanwhile, the sLe^x structure is involved in cell to cell adhesion (Dennis 1992), and type-2 chain structures may be involved in implantation of the blastocyst (Fenderson et al. 1991).

The infrequent and low expression of H-determinants in normal endometrium seem to be explained by a masking of this carbohydrate by its acting as a precursor-structure in synthesis of Le^y/Le^b and A antigens (Ravn et al. 1992a, b). These antigens are expressed with similar cyclic fluctuations in relation to the menstrual cycle and have the highest expression in late proliferative and mid-late secretory phases of the menstrual cycle (Ravn et al. 1992b, 1993). Meanwhile, atrophic endometria, which are characterized by low E_2 levels, showed a much higher expression of Le^y. These findings suggest that synthesis of H-determinants in the normal endometrium may be related to both E_2 actions, and as shown for stratified epithelia, to type of tissue maturation and differentiation (Dabelsteen et al. 1982, 1991).

In contrast to the very limited expression of H-antigen in normal endometrium, H-determinants showed an increased membraneous expression in endometrial carcinomas. Both Le^x and Le^y antigens showed a lowered membranous expression in adenomatous hyperplasias and endometrial carcinomas when compared with the high expression found in normal, atrophic endometrium, but a relative increase in cytoplasmic staining for Le^x, sLe^x, and Le^y. In a previous study, Le^y antigen was found to accumulate in endometrial carcinomas (Inoue et al. 1990). This discrepancy may be due to the very varied expression of both Le^y and Le^x antigens found in the normal endometrium in the present study. In fact, the endometrial carcinomas studied in the present study showed an increased membraneous expression of Le^y antigen when compared with the limited staining for Le^y in actively proliferating endometria. However, the carcinomas studied were, in general, associated with low serum levels of estrogen comparable to those found for women with an atrophic endometrium (Fig. 3).

Moreover, the correlation found between E_2 levels and expression of Le^x and Le^y antigens in the normal endometrium was lost in the malignant endometrium. Expression of H- and Le^y antigens in endometrial carcinomas were, in contrast with the findings in normal endometrium, related to the secretor status. These findings suggest that fucosyltransferases different from those catalysing synthesis in the normal endometrium may be involved in malignant endometrial cells. In addition to the Se-gene encoded α1-2 fucosyltransferase, it is suggested that one of several different variants of human α1–3 and α1–2 fucosyl-transferases with differences in acceptor specificity may be involved (Macher et al. 1991; Yazawa et al. 1993).

The changes found in adenomatous hyperplasia and endometrial carcinomas grade 1 were essentially similar, but tended to be less pronounced in adenomatous hyperplasias. When compared with grade 1 endometrial carcinomas, adenomatous hyperplasias showed less frequent H-antigen expression and less pronounced cytoplasmic accumulation of Le^y. This suggest that the tumour-associated changes in glycosylation take place early in neo-

plastic development. Therefore, H and Le^y expression seem to be of no value in the differential diagnosis of atypical hyperplasia versus grade 1 endometrial carcinoma.

The significance of the changes in glycosylation in endometrial carcinomas found in the present study are unknown. H/Le^y expression has been shown to correlate with survival in other carcinomas. Meanwhile, the expression of these carbohydrates showed no correlation with the FIGO stage. The glycosylation pattern is of importance for the activity of growth factors and their receptors (Dennis 1992), and both normal endometrial growth and glycosylation is hormonally influenced (Dallenbach-Hellweg 1987). Endometrial carcinomas retain, in general, receptors for estrogen and, less frequently, for progesterone (Nyholm et al. 1992). However, the hormonal dependency of cell-growth is lost in endometrial carcinoma cells. Our present results indicate that neoplastic transformation of endometrial cells may be accompanied also by a loss of a hormonal influence on expression of type-2 chain fucosylated carbohydrates.

In conclusion, the present study has substantiated the view that the expression of fucosylated type-2 chain carbohydrates relate to genetic and hormonal factors in the normal human endometrium. Endometrial carcinomas show, when compared with atrophic endometrium, a decrease in Le^x and Le^y antigen expression, and an increased expression of type-2 H antigen. These changes seem to be associated with a change in genetic and a loss of hormonal influence on expression of Le^y and Le^x antigens. This indicate that the tumour-associated changes in carbohydrate expression are due to specific tumour-associated qualitative and quantitative changes in the fucosyltransferases catalysing synthesis of fucosylated type-2 chain carbohydrates.

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