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Use of antibodies directed against blood group substances and lectins together with glycosidase digestion to study the composition and cellular distribution of glycoproteins in the large human airways

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ABSTRACT

Some 30 lectins in combination with glycosidase digestion and immunohistochemistry with 5 antibodies directed against antigens of the ABO and Lewis blood group systems were used to analyse the distribution and synthesis of glycoconjugates in the epithelium of the large airways in man. Both mucous gland cells and goblet cells were labelled by 12 of 30 lectins and by the antibodies, dependent on the ABO, Lewis, and secretor status. The corresponding binding patterns of the serous gland cells differed markedly from those of goblet and mucous gland cells and in general were not dependent on the ABO, Lewis, and secretor status. After digestion with neuraminidase and fucosidase, binding of soy bean agglutinin and peanut agglutinin to goblet and mucous gland cells was increased. Binding of peanut agglutinin to serous gland cells was stronger only after the digestion with neuraminidase. Digestion with O-glycosidase after the use of neuraminidase or fucosidase resulted in a decrease of peanut agglutinin binding to goblet and mucous gland cells. The present results show that the secretory products of goblet and mucous gland cells on the one hand and those of serous cells on the other differ considerably with respect to their terminal glycosylation. The glycosyltransferases coded by genes of the ABO and Lewis blood group and secretor systems are active only in goblet and mucous gland cells, resulting in the presence of the corresponding antigens. Precursor substances of blood group antigens types 1, 2, and 3 are found only in these cell types. In serous gland cells, blood group systems do not influence the glycosylation of glycoproteins. The results of the digestion with O-glycosidase indicates the presence of O-glycosylation in mucous gland and goblet cells, but not in serous gland cells.

Key words: Airways secretion; glycosylation; serous cell; mucous cell; goblet cell; mucin.

INTRODUCTION

Oligosaccharides are attached to proteins principally in 2 ways, O- or N-glycosylation (Hughes, 1983). In N-glycosylation, the bond between the oligosaccharide chain and the protein is established between N-acetyl-D-glucosamine and the amino nitrogen of L-asparagine (Hughes, 1983). In O-glycosylation, oligosaccharides are linked to proteins via N-acetyl-D-galactosamine to the hydroxyl groups of L-serine or L-threonine. Antigens of the ABO and Lewis blood group systems (Fig. 1) are found in the periphery of the O-linked oligosaccharides of human bronchial airways secretions (Bals et al. 1993). Mucins are the principal molecules glycosylated via O-glycosylation (Strous & Dekker, 1992). In the human tracheobronchial fluid, N- and O-glycosylated glycoproteins are found and have important functions both in health and disease (Lopez-Vidriero, 1981). Studies on the tissue distribution of these substances have been carried out at the mRNA, protein, and carbohydrate levels. Studies on the distribution of the protein component of secretory products (Mooren et al. 1982; Perini et al. 1989; Lee, 1993) and of the

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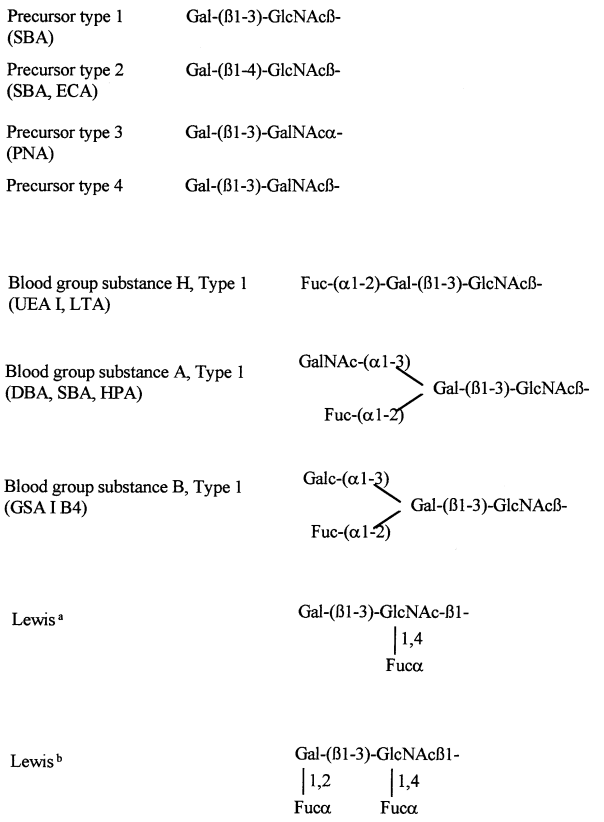

Fig. 1. Blood group antigens of the ABO and Lewis systems. Lectins that bind to these antigens in parenthesis (abbreviations of the lectins are explained in Table 1).

corresponding mRNA (Engelhardt et al. 1992; Audi et al. 1993; Dohrman et al. 1994; Van Seuningen et al. 1995) do not necessarily provide information about the cellular source of the mature, i.e. glycosylated, protein or about the glycosylation pathways in different secretory cell types. Other studies focused on the tissue distribution of carbohydrates of secretory products by means of immunohistochemistry and lectin histochemistry (Mazzuca et al. 1977, 1982; Schulte & Spicer, 1983; Spicer et al. 1983; Nishi et al. 1989; Castells et al. 1992). Nishi et al. (1989) localised ABO and Lewis antigens in mucous gland cells, Szulman (1960) found blood group antigens A and B in goblet and mucous gland cells of the airways. Ito et al. (1987, 1988, 1989*a*, *b*, 1990) and Nakajima et al. (1987, 1988) investigated the distribution of oligosaccharide structures and carbohydrate blood-group antigens in nonrespiratory tissue by means of immunohistochemistry and lectin histochemistry together with glycosidase digestion. The latter mentioned methods provided detailed insights into the cellular distribution of different glycoproteins and their synthesis. Corresponding investigations have not been carried out in respiratory tissue and knowledge about the tissue distribution of blood group antigens, precursor substances and active glycosyltransferase in the large human airways is not complete. However, several diseases are known to be associated with specific constellations of ABO, Lewis, and secretor constellations, e.g. asthma is significantly related to the nonsecretor phenotype (Kauffmann et al. 1996). Further, insight into the regulation of the synthesis of airways secretory glycoproteins with regard to the influence of ABO blood goup and secretor status is relevant to practical applications, such as in legal medicine. Finally, knowledge about the molecular biological differences between secretory cell types in the airways is important for understanding the processs of cell differentiation and its regulation in health and disease.

The aim of this study was to investigate the tissue distribution of terminal sugars, blood group antigens, precursor substances, and the cell type dependent synthesis pathways in the larger human airways by means of lectin and immunohistochemistry together with endo- and exoglycosidase digestions.

MATERIAL AND METHODS

Tissue collection, fixation, and embedding

Some 23 bronchi (main bronchus, lobar bronchus) were obtained from patients suffering from bronchial carcinoma. The material used for this study was macroscopically and microscopically healthy (no tumour infiltration, no signs of acute or chronic inflammation). The ABO and Lewis blood group status of the patients was assessed by routine blood agglutination methods. Their secretor status, i.e. whether the individuals secreted blood-group antigens into their saliva and some other secretions or not, was deduced from the immunohistochemical detection of ABO blood group antigens in the secretions or in secretory cells. The study included 8 patients with blood group A, 3 with B, 1 with AB, and 11 with O; 18 of these individuals were secretors, 5 were non secretors, 17 were Lewis positive, 6 were negative. The material was fixed in 5% formalin (in 0.1 M phosphate buffered saline, PBS) for 5 to 10 h at 4°C. After washing in PBS for 8 h at 4°C the tissue was dehydrated through a graded series of alcohol and xylene and embedded in paraffin.

Tissue processing and staining procedures

For staining or lectin or immunohistochemical procedures, serial sections of 5 µm thickness were

Table 1. *Lectins used in this study: name, abbreviation, source, specificity, and inhibitory sugar*

Lectin (abbreviation)	Source	Specificity	Inhibitory sugar
Canavalia ensiformis agglutinin (Con A)	*Canavalia ensiformis*	α-Man, α-Glc, α-GlcNAc	Man
Succinylated Canavalia ensiformis agglutinin (sCon A)	*Canavalia ensiformis*	α-Man, α-Glc, α-GlcNAc	Man
Lens culinaris agglutinin (LCA)	*Lens culinaris*	α-Man, α-Glc, α-GlcNAc, α-Fuk	Man
Pisum sativum agglutinin (PSA)	*Pisum sativum*	α-Man, α-Glc, α-GlcNAc, α-Fuc	Man
Griffonia simplicifolia agglutinin type II (GSA II)	*Griffonia simplicifolia*	β-GlcNAc	GlcNAc
Wheat germ agglutinin (WGA)	*Triticum vulgaris*	β-GlcNAc, sialic acid	GlcNAc
Datura stramonium agglutinin (DSA)	*Datura stramonium*	β-GlcNAc	GlcNAc
Aaptos papillata agglutinin (APA I)	*Aaptos papillata*	β-GlcNAc	GlcNAc
Solanum tuberosum agglutinin (STA)	*Solanum tuberosum*	β-GlcNAc	GlcNAc
Jacalin (Jac)	*Artocarpus integrifolia*	β-Gal	Gal
Maclura pomifera agglutinin (MPA)	*Maclura pomifera*	α-GalNAc; β-Gal	Gal
Euonymus europaeus agglutinin (EEA)	*Euonymus europaeus*	α-Gal	Gal
Griffonia simplicifolia agglutinin type I B4 (GSAI B4)	*Griffonia simplicifolia*	α-Gal	Gal
Viscum album agglutinin (VAA)	*Viscum album*	β-Gal	Gal
Ricinus communis agglutinin type I (RCA I)	*Ricinus communis*	β-Gal, β-GalNac	Gal
Peanut agglutinin (PNA)	*Arachis hypogaea*	β-Gal, Galβ(1–3) GalNAc	Gal
Erythrina cristagalli agglutinin (ECA)	*Erythrina cristagalli*	α-Gal, β-Gal, α-GalNAc, β-GalNAc	Gal
Soy bean agglutinin (SBA)	*Glycine max*	α-GalNAc	GalNAc
Dolichos biflorus agglutinin (DBA)	*Dolichos biflorus*	α-GalNAc	GalNAc
Sophora japonica agglutinin (SJA)	*Sophora japonica*	β-Gal	GalNAc
Wistaria floribunda agglutinin (WFA)	*Wistaria floribunda*	α-GalNAc, β-GalNAc	GalNAc
Helix pomatia agglutinin (HPA)	*Helix pomatia*	α-GalNAc; GalNac α(1–3)-GalNAc	GalNAc
Vicia villosa agglutinin (VVA)	*Vicia villosa*	α-GalNAc	GalNAc
Lotus tetragonolobus agglutinin (LTA)	*Lotus tetragonolobus*	α-Fuc	Fuc
Ulex europaeus agglutinin type I (UEA I)	*Ulex europaeus*	α-Fuc	Fuc
Limax flavus agglutinin (LFA)	*Limax flavus*	neuraminic acid	sialic acid
Limulus polyphemus agglutinin (LPA)	*Limulus polyphemus*	neuraminic acid	sialic acid
Sambucus nigra agglutinin (SNA)	*Sambucus nigra*	neuraminic acid	sialic acid
Phaseolus vulgaris agglutinin type L (PHA-L)	*Phaseolus vulgaris*	complex	—
Phaseolus vulgaris agglutinin type E (PHA-E)	*Phaseolus vulgaris*	complex	—

Fuc, fucose; Gal, galactose; GalNAc, N-acetyl-galactosamine; GlcNAc, N-acetyl-glucosamine; Man, mannose.

dewaxed and rehydrated. Histological and histochemical procedures applied were: haematoxylin/eosin (HE), periodic acid Schiff reaction (PAS), and Alcian blue at pH 1 and 2.5. For lectin staining, rehydrated sections were brought into 0.3% H_2O_2 (30 min, room temperature) to block endogenous peroxidase and rinsed in PBS containing 0.01 M $CaCl_2$ and $MgCl_2$, pH 7.4 for 10 min at room temperature (RT). The lectins used in this study (Table 1) were conjugated with biotin and were obtained from Medac (Hamburg, Germany) or Sigma (Deisenhofen, Germany). The sections were incubated with solutions of the lectins for 1 h at RT. After washes in PBS (3×5 min) the sections were transferred to a solution of avidin-peroxidase-complex (30 µg/ml, Sigma) for 30 min at RT. After washes in PBS, the binding sites of lectins were visualised by 0.05% 3,3′-diaminobenzidine (DAB) and 0.003% H_2O_2 in PBS for 10 min at RT. Some of the sections were counterstained with haematoxylin. The sections were washed, dehydrated and mounted in DEPX. Control experiments were carried out by incubation with the lectins together with the corresponding inhibitory sugar (0.1 M). Sections were viewed under an Olympus BH-2 microscope, and micrographs taken with an Agfapan APX 25 film.

Table 2. *Antibodies used in this study: name, clone name, source, isotype, and working dilution*

Monoclonal antibody	Clone name	Source/ isotype	Working dilution
Anti-blood group substance A	81 FR 2.2	mouse/IgM	1:300
Anti-blood group substance B	3 E 7	mouse/IgM	1:300
Anti-blood group substance H	92 FR A2	mouse/IgM	1:300
Anti-blood group substance Le^a	78 FR 2.3	mouse/IgM	1:100
Anti-blood group substance Le^b	96 FR 2.10	mouse/IgM	1:100
Anti-IgM mouse secondary antibody	—	goat/IgM	1:500

Table 3. *Glycosidases for the digestions and conditions of incubation*

Enzyme (source)	Condition of incubation
Neuraminidase (*Arthrobacter ureafaciens*)	0.25 U/ml; acetate buffer, 50 mM pH 5, 6–24 h at 37 °C
O-glycosidase (*Diplococcus pneumoniae*)	0.17 U/ml, phosphate buffer, 0.05 M pH 7, 20 h at 37 °C
β-galactosidase (*E. coli*)	2 U/l, citrate phosphate buffer, 0.2 M pH 8, 20 h at 37 °C
α-galactosidase (green coffee bean or cloned in *E. coli*)	2 U/l, $(NH_4)_2SO_4$ solution, 3.2 M pH 6, 24 h at 37 °C
L-fucosidase (bovine kidney)	1 U/l, citrate phosphate buffer, 0.1 M pH 5, 20 h at 37 °C

For the immunohistochemistry we used an indirect technique. After blocking the endogenous peroxidase as described above and rinsing in PBS, the sections were treated with 1% goat serum in PBS (30 min, RT). Then they were incubated with the primary antibody for 1 h at RT. After washes in PBS, the sections were incubated with a peroxidase-conjugated goat antibody directed to mouse-IgM for 1 h at RT. After further washes in PBS, the binding sites of the antibodies were visualised with DAB as described above. Some of the sections were counterstained with haematoxylin. Finally the sections were washed, dehydrated and mounted in DEPX. Control experiments were carried out by omitting the first antibody or both antibodies. The antibodies used, together with their clone number, source, isotype, and working dilutions are summarised in Table 2. They were purchased from DAKO (anti-A, B, and H), from Sigma (antimouse) or from BAG (Lich, Germany; anti-Le^a and anti-Le^b).

Incubation with glycosidases

Dewaxed sections were washed in PBS and brought into the corresponding enzyme buffer for 15 min. A solution of the enzyme was applied to the tissue and the digestion was performed as described in Table 3. After washes in PBS, the sections were stained with the lectins GSA I B4, SBA, and PNA as described above. After digestion with neuraminidase and fucosidase some of the sections were additionally treated with O-glycosidase before lectin staining was performed. Control experiments were carried out by incubating tissue sections with buffer and omitting the enzyme. The enzymes were purchased from Boehringer Mannheim (Mannheim, Germany).

Cell type recognition and analysis of stained tissue

The secretory cell types were identified by their morphology and localisation, or their staining patterns with histological and histochemical staining procedures (HE, PAS, Alcian blue) in the next serial section: mucous cells of the glands were identified by their basal, usually flat and heterochromatin rich nuclei and prominent cell boundaries and were stained by PAS and Alcian blue at pH 1. Serous cells of the glands were identified by their round, central nuclei and lack of prominent cell boundaries and were stained by PAS, but not by Alcian blue at pH 1. Goblet cells, which occur in the respiratory surface epithelium, were positive with PAS and Alcian blue at pH 1, their distended apical regions were filled with many secretory vesicles. The results were obtained by viewing 3 sections per individual for each lectin, antibody, or digestion. On each section, 300 secretory cells of each type were analysed. The determination of the quantity of the staining intensity was based on a semiquantitative subjective score from − (no binding) to +++ (strong binding).

RESULTS

Lectin and immunohistochemistry of goblet and mucous gland cells

Mucous cells of the glands and goblet cells of the surface epithelium showed similar results. The lectins WGA, Jac, MPA, GSA I B4, VAA, RCA I, PNA, ECA, SBA, DBA, WFA, HPA, VVA, LTA, UEA I,

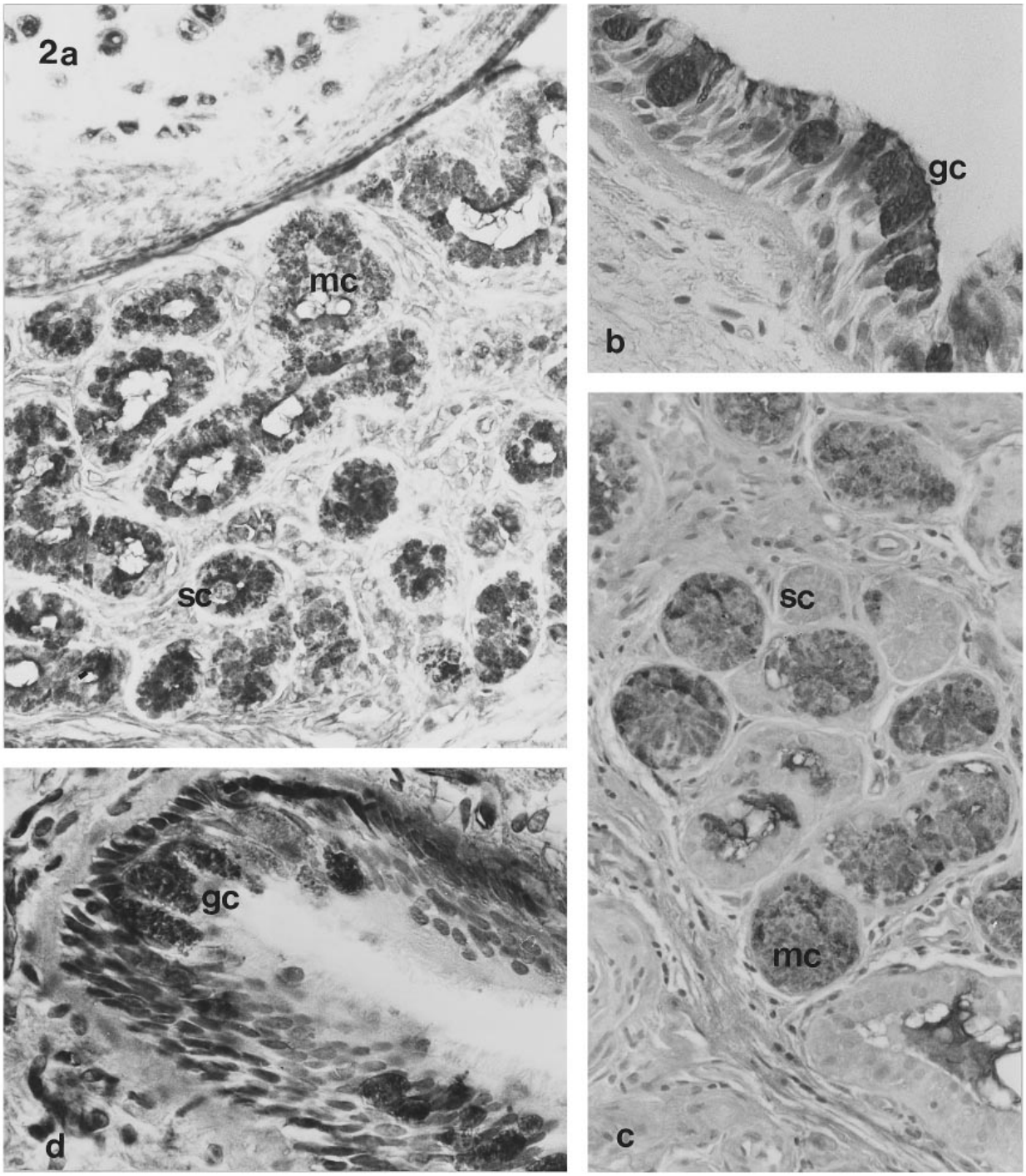

Fig. 2. Goblet and mucous gland cells stained with biotin-conjugated lectins. sc, serous gland cell; mc, mucous gland cell; gc, goblet cell. (A) Tissue from a non secretor individual stained with WGA. ×315; (B) goblet cells from a secretor individual with blood group A stained with DBA, ×525; (C) tissue from a secretor individual with blood group B stained with GSA I B4; haematoxylin counterstain, ×525; (D) goblet cells from a secretor individual with blood group B stained with HPA; haematoxylin counterstain, ×525.

LFA, LPA, SNA, EEA, and APA bound to these cells (Fig. 2). Some of the lectins bound only in the tissue of secretors dependent on the ABO blood group (Fig. 2*b*, *c*): blood group A (SBA, DBA, HPA), B (GSA I B4), AB (SBA, DBA, HPA, GSA I B4). In secretors, UEA I and LTA bound consistently, and for blood group O these 2 lectins bound particulary strongly. In nonsecretors, the lectins WGA, GSA I B4, RCA I, PNA, and WFA bound more strongly than in secretors (Fig. 2*a*). In secretors, binding for the antibodies directed to ABO blood group substances was dependent on the ABO blood group (Fig. 4*a*). Antibodies to Le^a or Le^b bound to goblet and mucous gland cells only in Lewis positive individuals (Fig. 4*b*).

Table 4. *Goblet and mucous gland cells: results of lectin and immunohistochemistry**

Lectins/antibodies		Blood group A secretor	Blood group B secretor	Blood group O secretor	Nonsecretor
Blood group A and GalNAc-specific substances					
	anti-A	90%+++	—	—	—
	DBA	95%+++	5%++	5%++	—
	SBA	95%+++	50%+basal	50%+basal	—
	HPA	50%++	50%++basal	50%++basal	50%++basal
	SJA	80%++	80%++	80%++	80%++
	WFA	50%+	50%+	50%+	50%+
	VVA	40%+	40%+	40%+	40%+
Blood group B and Gal-specific substances					
	anti-B	—	90%+++	—	—
	GSA I B4	—	95%+++	—	20%+
	Jac	50%+	50%+	50%+	50%+
	MPA	90%++	90%++	90%++	90%++
	EEA	100%+++	100%+++	100%+++	100%+++
	VAA	50%++	50%++	50%++	50%++
	RCA I	20%++	20%++	20%++	80%++
	PNA	20%+basal	20%+basal	20%+basal	80%++
	ECA	40%++	40%++	40%++	40%++
Blood group O and Fuc-specific substances					
	anti-H	50%+	50%+	80%+++	—
	LTA	10%+	10%+	95%++	—
	UEA I	10%+	10%+	95%++	—
Lewis antigens-specific antibodies					
	anti-Le^{a}**	80%+++	80%+++	80%+++	80%+++
	anti-Le^{b}**	80%+++	80%+++	80%+++	—
Man-specific lectins					
	Con A	20%+	20%+	20%+	20%+
	sCon A	20%+	20%+	20%+	20%+
	LCA	—	—	—	—
	PSA	—	—	—	—
GlcNAc-specific lectins					
	GSA II	—	—	—	—
	WGA	65%+++	65%+++	65%+++	100%+++
	DSA	10%+	10%+	10%+	10%+
	APA	80%++	80%++	80%++	80%++
	STA	—	—	—	—
Sialic acid and complex sugar-specific lectins					
	LFA	60%++	60%++	60%++	60%++
	LPA	70%++	70%++	70%++	70%++
	SNA	70%++	70%++	70%++	70%++
	PHA-L	—	—	—	—
	PHA-E	—	—	—	—

* Percentages indicate the part of cells that are labelled by the lectin or antibody; the intensity of staining is based on a semiquantitative score ranging from — (no staining) to +++ (strong staining).
** Binding of antibodies to Le^{a} or Le^{b} was detected only in tissue from Lewis positive individuals; secretor individuals with blood group AB showed same staining pattern as individuals with blood group A and B. Abbreviation as in Table 1.

Dependent on the secretor status, only Le^{a} (non secretors) or Le^{a} and Le^{b} (secretors) could be detected. The results are summarised in Table 4.

Lectin and immunohistochemistry of serous gland cells

The binding of the lectins to serous cells did not show any dependency on the secretor, ABO, or Lewis blood group status. Binding was demonstrated for the lectins ConA, sConA, LCA, PSA, WGA, PNA, HPA, LTA, UEA I, LFA, LPA, SNA, and APA (Fig. 3*a*, *b*). Only the antibody to H antigen bound to the serous cells, to 10% of them in the apical part of their cytoplasm (Fig. 4*a*); none of the other antibodies showed binding. The results are summarised in Table 5.

Control experiments showed no false positive results in the secretory cells (Fig. 3*c*).

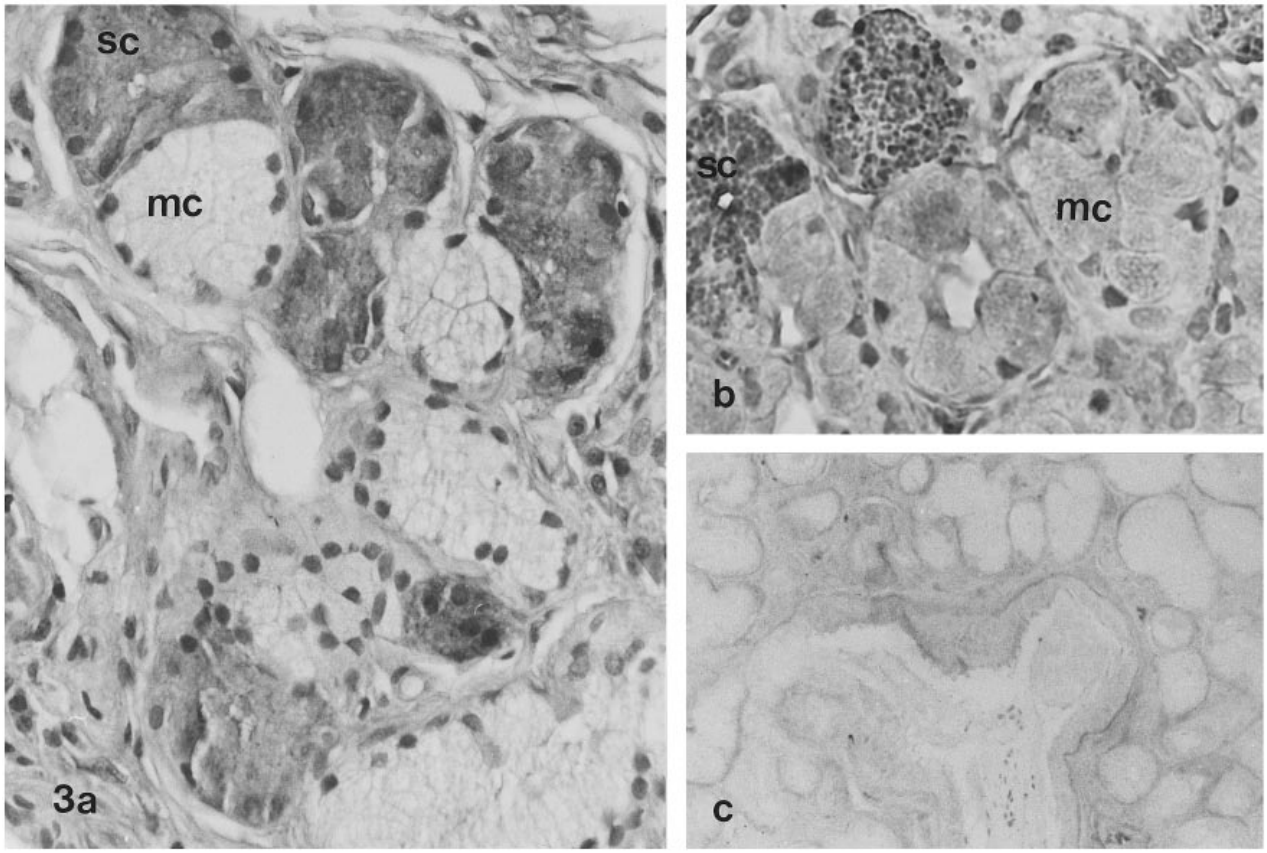

Fig. 3. Serous gland cells stained with lectins. sc, serous gland cells; mc, mucous gland cells. (A) Staining with LCA, haematoxylin counterstain, × 750; (B) staining with ConA; haematoxylin counterstain, × 525; (C) control section incubated with ConA together with the inhibitory sugar D-Man, × 315.

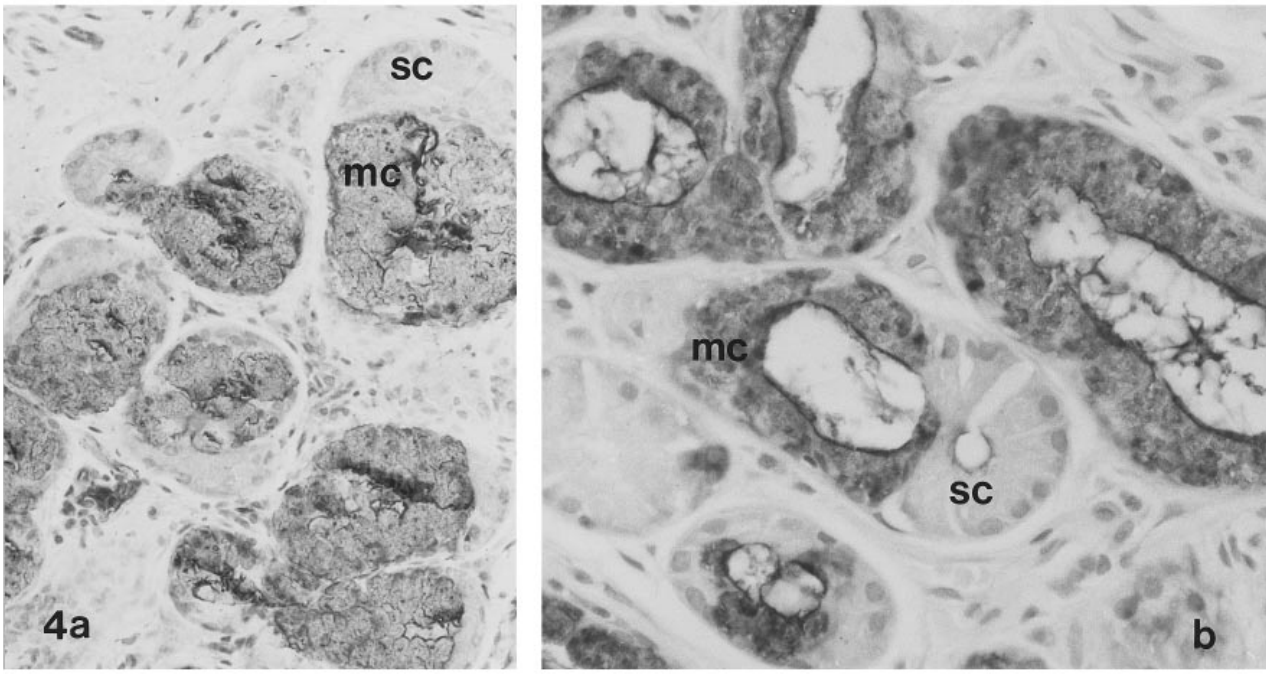

Fig. 4. Binding of antibodies to serous (sc) and mucous (mc) gland cells. (A) Anti-H binding to mucous gland cells and to the apical part of serous gland cells, tissue from a secretor-individual with blood group O; haematoxylin counterstain, × 750; (B) anti-Le^b binding to mucous gland cells, tissue from a secretor-individual with blood group A; haematoxylin counterstain, × 750.

Table 5. *Serous cells of the glands: results of lectin and immunohistochemistry.*

Lectins/ antibodies	Secretor	Nonsecretor
Con A	100% ++	100% ++
sCon A	100% ++	100% ++
LCA	100% ++	100% ++
PSA	100% ++	100% ++
GSA II	—	—
WGA	40% ++	40% ++
DSA	—	—
APA	80% +	80% +
STA	10% +	10% +
Jac	—	—
MPA	—	—
EEA	—	—
GSA I B4	—	—
VAA	—	—
RCA I	10% +	10% +
PNA	40% + apical	40% + apical
ECA	10% +	10% +
SBA	—	—
DBA	—	—
SJA	—	—
WFA	10% +	10% +
HPA	50% +	50% +
VVA	—	—
LTA	40% + apical	—
UEA I	40% + apical	—
LFA	100% ++	100% ++
LPA	50% +	50% +
SNA	50% +	50% +
PHA-L	—	—
PHA-E	—	—
anti-A	—	—
anti-B	—	—
anti-H	10% + apical	—
anti-Le^a	—	—
anti-Le^b	—	—

Percentages indicate the part of cells that are labelled by the lectin or antibody; the intensitiy of staining is based on a semiquantitative score ranging from — (no staining) to +++ (strong staining).

Results of the enzyme digestion procedures and lectin histochemistry

Only tissue of secretors was used for the digestion experiments. Compared with the staining of secretory cells using PNA without any further treatment (Fig. 5*a*), the use of neuraminidase resulted in increased binding of PNA to serous and mucous gland cells and to goblet cells (Fig. 5*b*). Additional digestion with the O-glycosidase decreased the reactivity of PNA in goblet and mucous gland cells, but not in serous gland cells (Fig. 5*c*). Digestion with fucosidase had similar effects on the binding patterns of PNA as did digestion with neuraminidase. The binding of SBA to goblet and mucous gland cells was increased by digestion with neuraminidase (Fig. 5*d*) and fucosidase. The α-galactosidase increased the binding of SBA and PNA to goblet and mucous gland cells, but only in blood group B tissue. Further, binding of GSA I B4 was reduced by digestion with α-galactosidase in blood group B tissue. The β-galactosidase did not influence the binding patterns of the lectins. Controls omitting the enzymes and using only the buffer revealed no detectable change in the lectin staining patterns. The results of the digestions followed by lectin histochemistry are summarised in Table 6.

DISCUSSION

The aim of this study was to investigate the cell type dependent glycosylation of secretory glycoproteins and synthesis pathways by means of lectin and immunohistochemistry together with endo- and exoglycosidase digestions. The results of the incubations with lectins, antibodies, with or without glycosidase digestion, together with known ABO, secretor, and Lewis blood group status, allow the detailed analysis of cell-type specific terminal sugars, oligosaccharides, blood group specific precursors, and mature antigens. Further, from these results the active glycosyltransferases and the corresponding coding genes can be deduced.

Mucous and serous gland cells and goblet cells could easily be distinguished by their localisation and morphology. Whereas mucous and serous gland cells differed significantly in their staining patterns, the goblet and mucous gland cells showed identical results. This indicates that goblet and mucous gland cells appear to be closely related and produce similar secretory products. Different staining patterns between these 2 cell types as found by Mazzuca et al. (1977, 1982) were not observed. The fact that not all mucous and serous gland cells or goblet cells were labelled by a single lectin or antibody need not necessarily indicate a heterogenity of the cell populations, but may indicate different stages of maturation. Nakajima et al. (1988) described different lectin staining patterns in cells of different maturities in human salivary glands.

According to publications on the sugar-specificities of lectins (see Wu et al. 1988) the cell type specific terminal sugars can be deduced from the lectin staining patterns. In goblet and mucous gland cells, the following terminal sugars are present, independent of the secretor status or the ABO blood group: α-L-fucose, β-D-galactose, β-N-acetyl-D-galactosamine, β-acetyl-D-glucosamine, and neuraminic acid. Dependent on the ABO-blood group, in secretors α-N-acetyl-D-galactosamine (blood group A individuals)

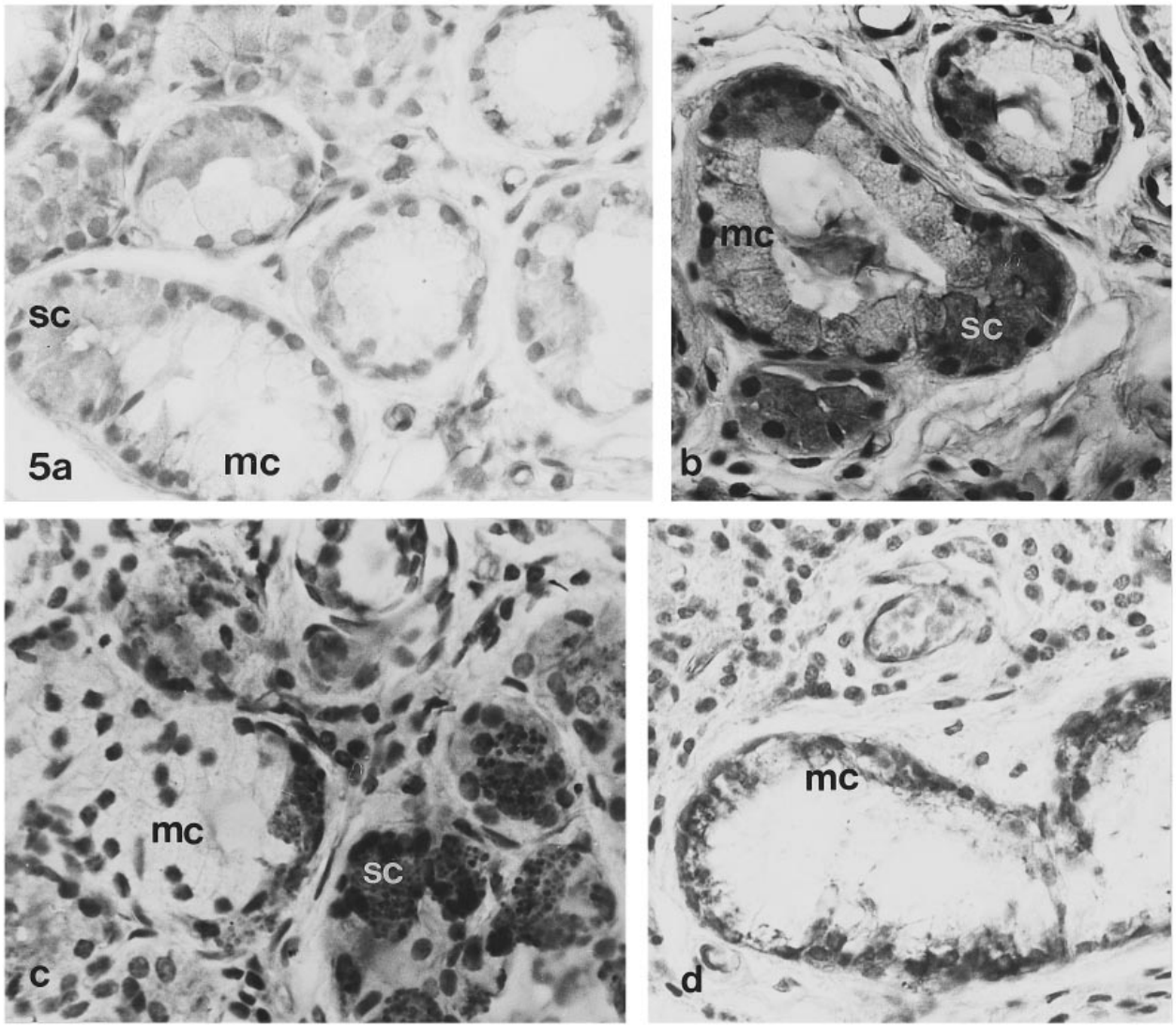

Fig. 5. Effects of glycosidase digestion on lectin staining of secretory cells. sc, serous gland cells; mc, mucous gland cells. All sections counterstained with haematoxylin. (A) Gland cells stained with PNA, ×525; (B) gland cells stained with PNA after digestion with neuraminidase, ×525; (C) gland cells stained with PNA after digestion with neuraminidase and O-glycosidase, ×525; (D) gland cells stained with SBA after digestion with neuraminidase, ×525.

and α-D-galactose (blood group B individuals) can be found; in tissue from individuals with blood group AB both sugars are present. α-D-mannose is not abundant in goblet or mucous gland cells as indicated by the absence of binding sites for mannose-specific lectins. In serous gland cells, however, terminal D-mannose was found. Further, α-L-fucose, β-D-galactose, β-acetyl-D-glucosamine, and neuraminic acid were detected in this cell type. These conclusions with regard to the distribution of terminal sugars in secretory cells of human airways are in agreement with the results of other authors (Mazzuca et al. 1977, 1982; Spicer & Schulte, 1983, 1985). Since the specificities of lectins are not directed to a single terminal sugar, more than one lectin always has to be used for a valid interpretation of the results. In the present study, we used a large number of lectins to additionally support the conclusions drawn from the lectin histochemical studies mentioned above which used a smaller panel of lectins.

The results deduced from lectin histochemistry not only provide information on the cell type specific distribution of terminal sugars, but also allow speculations on the entire secretory products. As indicated by former lectin histochemical results (Mazzuca et al. 1982), goblet and mucous gland cells seem to produce high molecular weight mucins, whereas serous gland cells seem to produce low molecular weight N-glycosidic built glycoproteins. The results of the present study confirm these correlations. Secretory products of goblet and mucous gland cells carry ABO blood group antigens and terminal N-acetyl-D-

Table 6. *Results of lectin histochemistry with SBA and PNA after glycosidase digestion.*

Serous gland cells	Treatment: None	Fucosidase	Neuraminidase	α-Galactosidase	Neuraminidase ↓ O-Glycosidase	Fucosidase ↓ O-Glycosidase
PNA	+ap	+ap	+++ap	+ap	+++ap	+ap
SBA	—	—	—	—	/	/
Mucous gland cells and goblet cells	**Treatment: None**	**Fucosidase**	**Neuraminidase**	**α-Galactosidase**	**Neuraminidase ↓ O-Glycosidase**	**Fucosidase ↓ O-Glycosidase**
PNA	+ba	+++	++ba	+++ba in B, AB	+ba	+ba
SBA	+++A, AB	+ and A, AB	+++ba and A, AB	+++ba and A, AB	/	/

ap, apical; ba, basal; A, B, AB, staining only in tissue from blood group A, B, or AB individuals; —/+/++/+++ = staining score: no staining/weak/moderate/strong staining; /, not tested

galactosamine, but do not have abundant D-mannose. These are characteristics of O-glycosidically built mucins (Strous & Dekker, 1992). Furthermore, digestion with O-glycosidase, which removes O-linked sugars from the protein, only changes lectin-staining patterns of goblet and mucous gland cells. The secretory products of serous cells carry D-mannose, but do not have N-acetyl-D-galactosamine as a terminal sugar, as is typical for N-glycosidically built glycoproteins (Hughes, 1983). The results allow well founded, but not definitive conclusions on the entire molecules, since lectins or antibodies only give information on the terminal sugars.

The results of the lectin histochemistry indicate that cells that have identical staining patterns as the serous gland cell do not exist in the surface epithelium of the larger human airways. Thus serous cells as described by Rogers et al. (1993) in the surface epithelia of the small human airways do not exist in the large airways.

In general, the presence of antigens of the ABO and Lewis systems is restricted to the mucous gland and goblet cells, the H antigen is to be found only in few serous gland cells. This may indicate that the anti-H binding serous cells represent an intermediate cell type between the mucous and the serous cell type. The lectins DBA, SBA, GSA I B4, HPA, UEA I, and LTA bind to terminal sugars that are part of the different ABO blood group antigens, since their staining patterns are associated with the binding of the corresponding antibody or the ABO status. This is additionally demonstrated by the decrease of staining intensity of GSA I B4 after removal of the terminal α-Gal by the digestion with α-galactosidase in blood group B tissue.

Analysing the results of lectin histochemistry following the glycosidase digestions, the distribution of oligosaccharides and precursors for the synthesis of blood group antigens can be deduced. Several types of precursor oligosaccharides are known that are glycosylated by glycosyltransferases coded by genes of the ABO, Secretor, and Lewis systems to produce different blood group antigens (Fig. 1). These precursors can be detected and localised by means of lectin histochemistry together with glycosidase digestion, as shown by Ito et al. (1987, 1988, 1989*a*, *b*, 1990) and Nakajima et al. (1987, 1988) in non respiratory tissue. SBA binds to galactose-(β1-3,4)-N-acetyl-D-glucosamine-R (precursors type 1 and 2) (Ito et al. 1987) and PNA can be used to detect O-glycosidically serine/threonine-linked galactose-(β1-3)-N-acetyl-D-galactosamine (precursor type 3) (Ito et al. 1988). Further, ECA is specific for type 2 precursor (Ito et al. 1990). Without enzymatic digestion, the binding of SBA and PNA to the secretory cells was weak or absent, except for the binding of SBA in blood group A and AB tissue. After the removal of the terminal L-fucose or neuraminic acid by digestion with fucosidase or neuraminidase, the increased binding of PNA and SBA to mucous gland cell or goblet cells indicates the presence of the corresponding oligosaccharide structures. Since the O-glycosidase removes galactose-(β1-3)-N-acetyl-D-galactosamine O-glycosidically bound to L-serine or L-threonine of the protein, the decrease of PNA

staining after digestion confirms the presence of the PNA reactive oligosaccharide O-linked to the protein. The results of this study indicate the presence of precusor types 1, 2, and 3 in goblet and mucous gland cells. The results of the digestions with neuraminidase and fucosidase indicate that L-fucose or neuraminic acid are added as terminal sugars to the precursors in both cell types. The binding of PNA in serous gland cells after digestion with fucosidase or neuraminidase does not indicate the presence of protein-linked precursor type 3, since the digestion with O-glycosidase did not alter the binding of PNA. The binding of PNA may be due to D-galactose that can be detected by the lectin after the terminal sugar has been removed by the enzyme.

The results of the lectin and immunohistochemistry, together with the known ABO and Lewis blood group status, permit analysis of the cell type specific pathways of oligosaccharide synthesis, the distribution of glycosyltransferases, and the corresponsing coding genes. In goblet and mucous gland cells, the synthesis of ABO and Lewis blood group antigens is known to be dependent on secretor status (Szulman, 1960; Nishi et al. 1989). The results of this study indicate that in these cells the precursors type 1, 2, and 3 are present and are fucosylated by the glycosyltransferase coded by the *Se* gene of the secretor system. The glycosylation by the *Se* coded transferase results in the H antigen that is further modified by glycosyltransferases coded by the *A* and *B* alleles of the ABO blood group system, resulting in the A or/and B antigens. In goblet and mucous gland cells, the glycosyltransferase coded by the *Le* gene fucosylates the H (type 1) antigen resulting in the Le^b antigen or fucosylates the precursor type 1 resulting in Le^a antigen. In secretors, the *Le* coded glycosyltransferase acts on the H (type 1) antigen as well as on the precursor substance and thus Le^a and Le^b could be detected. In nonsecretors, only the precursor type 1 substance can be fucosylated, thus the Le^b antigen is absent. These results fit well into the current concepts about the genetics of the blood group systems (Oriol et al. 1986). Figure 6 summarises the cell type specific synthesis pathways of blood-group antigens.

The results of this study allow new insight into the cell-type specific synthesis pathways of the carbohydrate moieties of human tracheobronchial glycoproteins and into the relationship of differentiated secretory cells. To study the cell type specific pathways of synthesis, the in situ approach is neccesary, since it is yet not possible to isolate pure cell populations of a single secretory cell type by means of cell culture techniques. Serous gland cells on the one hand and goblet and mucous gland cells on the other represent the 2 principal secretory cell types in the larger airways in man.

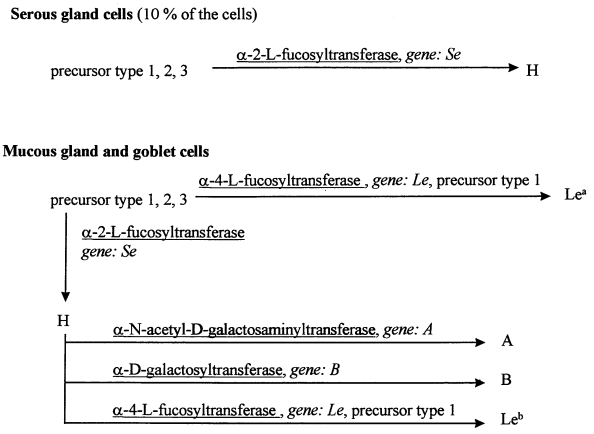

Fig. 6. Cell type specific distribution of blood group antigens, glycosyltransferases and corresponding coding genes.

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