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Modulation of the glycoconjugate expression in the tracheo-bronchial epithelium during sustained hypovitaminosis A

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Summary. The aim of this study was to determine the influence of sustained marginal vitamin A deficiency on the morphology of glycoconjugate expression in the tracheobronchial epithelium of guinea pigs. The distribution of oligosaccharide chains was investigated by applying a panel of 24 lectins. Glycosaminoglycans were detected by histochemical techniques. Number as well as morphology of ciliated cells showed no significant alterations in hypovitaminosis A. In contrast, the quantity of goblet cells was constantly decreased. A considerable reduction of secretory granules was also observed in these cells. Cytomembranes of ciliated cells (especially in the area of ciliar extensions) showed constant alterations in the patterns of lectin binding in vitamin A-depleted guinea pigs. Our results demonstrate a significant augmentation of accessibility of fucosyl molecules in proximal domains of glycoconjugates of ciliary membranes, whereas the presence of mannose structures seemed unchanged. In distal bronchioli, terminal N-acetylgalactosamine molecules were expressed. During marginal vitamin A deficiency, ciliary cells were specially labelled by GSA I_B, indicating presentation of terminal galactose molecules in α-position. Additionally, the cytoplasm of epithelial cells demonstrated enhanced concentrations of polyantennary oligosaccharide core structures. Staining of epithelial cells by VVA was restricted to control specimens. Abundance of N-acetylglucosamine residues on the non-reducing terminus of oligosaccharides was significantly enhanced in the connective tissue of depleted animals as demonstrated by the binding patterns of GSA II. We suggest that altered oligosaccharide patterns may contribute to enhanced predisposition to tracheobronchial infection in marginal vitamin A deficiency.

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Introduction

Glycosylation of proteins or lipids occurs ubiquitously in living organisms. The proteins can contain oligosaccharide chains of 1 to 10 sugars that are covalently connected to specific amino acid sequences (Lis and Sharon, 1993; Akif et al., 1994). They can be grouped into linear sugar molecules that are O-glycosidically linked to the hydroxyl group of serine or threonine and complex, branched carbohydrates that are N-glycosidically bound to asparagine (Parekh, 1991). Recent advancement in analytic technology has revealed that many of these carbohydrate chains play important roles as signals for biological information transfer (Kobata, 1992). Glycosylation is modulated by species and organ-specific differences (Goochee and Monica, 1990). In addition, accumulation of information on the structural characteristics of carbohydrates has further revealed that hormonal as well as nutritional influences can modify activity of the glycosylation machinery (Varki, 1993; Akif et al., 1995). The enormous resulting structural heterogeneity that complicates unequivocal analysis is the main reason for the rather fragmentary availability of information in glycobiology (Laine, 1994). This potential for variation by modulation can enable the elucidation of characteristic modifications in the expression of carbohydrate molecules. They can serve as sensitive markers of deviations from the normal status accompanying or even preceeding morphological alterations of epithelial cells (Sumar et al., 1993).

The influence of retinoids on glycoconjugate metabolism has so far not been extensively investigated. In a recent biochemical investigation, for example it has been demonstrated that exposure to vitamin A alters glycoconjugate synthesis in vitro (Guma and Bernard,

1994). Preliminary results further indicate that catabolism of carbohydrates is also affected (Braunhut and Moses, 1994). As a consequence of these intricate mechanisms, retinoids appear to quantitatively or qualitatively modify expression of sugar structures of cellular and extracellular components (Chen et al., 1994). Lung tissue exhibits a high level expression of retinoid receptors (Grummer et al., 1994) and it has been convincingly demonstrated in tissue and cell culture that mucin production of bronchial epithelium depends significantly on retinoic acid availability (Manna et al., 1994). In addition, in a more advanced stage of vitamin A deficiency, mucus-secreting cells are typically replaced by squamous epithelium (McDowell et al., 1984). However, respiratory infections are significantly enhanced in marginal vitamin A deficiency when no characteristic alterations in tracheobronchial morphology can be detected (Pinnock et al., 1986). The underlying causes are at present not completely understood. Therefore, on the basis of the possibility of the modulatory response, we felt encouraged to propose that sustained marginal vitamin A deficiency may induce subtle, but characteristic modifications in the glycoconjugate expression preceding squamous metaplasia. This structural alteration, which can be completely reversed by vitamin A, is said to be an early stage of carcinogenesis (Lotan et al., 1984). consequently, it does not seem to be surprising that a low intake of vitamin A or its provitamin beta-carotene is associated with increased morbidity of cancers of the lung.

The most sensitive morphological probe to analyze differences in oligosaccharide composition is the application of lectin histochemistry. Lectins are carbohydrate-binding molecules, classified according to their specificity for monosaccharides, that are usually situated in a terminal position (Damjanov, 1987). Only few lectins detect corresponding sugar molecules irrespective of contiguous carbohydrate arrangement. Most reveal preferential affinity only if distinct prerequisites of neighborhood (stereochemical and sequence requirements) are satisfied. The most important parameters are anomeric specificity α-/ß-position) and linear or branched glycoconjugate arrangement, respectively. Other sugar receptors display extended binding sites for di- or trisaccharides (Spicer and Schulte, 1992). Lectins with more stringent requirements usually identify significantly fewer structures than those with a lower degree of specificity. Incubation of serial sections with a battery of lectins that represent the same nominal carbohydrate affinity, but with different binding specificities is therefore of potential value for determining the extent of presence of characteristic oligosaccharide structures in tissue sections (Danguy et al., 1994). In histopathology it has been reported that lectin histochemistry is able to differentiate preneoplastic conditions even in cells with apparently normal appearance in relation to healthy tissue (Bresalier et al., 1985). In several studies the lectin binding patterns of tracheobronchial specimens have been thoroughly analyzed. These investigations documented impressive modulations in the staining results during fetal development (Castells et al., 1991), aging, inflammation (Kasper et al., 1993), and tumor development (Kawai et al., 1988; Shiba et al., 1984), respectively. However, to our knowledge the influence of vitamin A metabolism on glycomorphology of tracheobronchial tissue in vivo has not been determined up to now. In order to reveal the structural alterations of glycoconjugates, a panel of 24 plant lectins was applied in vitamin A-depleted guinea pigs and control animals. To elucidate possible species-dependent differences, results were compared with healthy lung specimens of rats and mice. Our findings indicate that the retinoid status characteristically influences the lectin-binding patterns. As a cautionary note for interpretation of the results it has to be emphasized that species-dependent differences have to be carefully considered.

Materials and methods

Breeding conditions

A total of 125 guinea pigs (Himalayan spotted; Biological Research Laboratories LTD, CH-4414 Füllinsdorf, Switzerland) were kept under constant hygienic conditions at a room temperature of 25 °C at 17-fold air circulation/h and at 50% relative humidity. To avoid access to uncontrolled retinol source originating from mother's stores, females were already in a state of latent vitamin A deficiency at the beginning of gestation. From birth on animals were fed a powdered purified diet which contained all the essential nutrients apart from vitamin A (Biesalski and Stofft, 1992). Control animals additionally received retinyl acetate (100 IU/day in oily solution) by pharyngeal tube. The weight curve was taken as criterion for normal development.

Determination of retinoid metabolism

Serum retinoid and liver retinyl ester concentrations were analyzed by means of HPLC at weekly intervals (Fig. 1; Biesalski and Weiser, 1990). Animals reached their body weight growth plateau phase (indicating beginning of deficiency) at about day 100. On day 104, 15 vitamin A-deficient and 7 pair-fed control animals were selected, anesthetized with Nembutal (2 mg/kg body weight) and decapitated. The vitamin A content of serum and selected tissues of these animals was determined.

Morphological investigations

Specimens were resected from standardized regions: trachea (5-7 cartilage units proximal to the carina), main bronchi, lung tissue. Biopsies were immediately immersion-fixed in phosphate buffered formaldehyde(4%) or in Bouin's fluid. Fixation procedures were chosen after the effects of various

fixatives, as used in a corresponding study (Allison, 1987). The extent of subsequent lectin binding was systematically evaluated in two normal fed guinea pigs (day 78). Specimens were routinely dehydrated with graded propanol, cleared in xylene and embedded in paraffin. Serial 7 µm-thick sections were cut,

Table 1. Lectin characteristics.

NAME	ABBREVIATION	CARBOHYDRATE BINDING AFFINITY
Canavalia ensiformis	Con A	α/ß-Man > α-Glc > α-GlcNAc (especially branched structures)
Lens culinaris	LCA	α/ß-Man > α-Glc > α-GlcNAc (fucosylated core region; bi-/triantennary oligosaccharides)
Pisum sativum	PSA	α/ß-Man > α-Glc = α-GlcNAc (fucosylated core region; bi/triantennary oligosaccharides)
Lycopersicum esculentum	LEA	GlcNAc-[(ß1,4)-GlcNAc]$_{1-3}$
Solanum tuberosum	STA	GlcNAc-[(ß1,4)-GlcNAc]$_{1-4}$
Datura stramonium	DSA	GlcNAc-[(ß1,4)-GlcNAc]$_{1-3}$ = Gal-(ß1,4)-GlcNAc
Griffonia simplicifolia II	GSA II	terminal GlcNAc = glycogen
Triticum vulgare	WGA	GlcNAc-[(ß1,4)-GlcNAc]$_{1-2}$ > terminal [ßGlcNAc]$_n$ > Neu5Ac
Triticum vulgare	sWGA	GlcNAc-[(ß1,4)-GlcNAc]$_{1-2}$ > terminal ßGlcNAc
Erythrina cristagalli	ECA	Gal-(ß1,4)-GlcNAc
Ricinus communis	RCA	Gal-(ß1,4)-GlcNAc > terminal ß-Gal > terminal α Gal
Phaseolus vulgaris E	PHA-E	Gal-(ß1,4)-GlcNAc-(ß1,2)-man (bisected complex oligosaccharides)
Phaseolus vulgaris L	PHA-L	Gal-(ß1,4)-GlcNAc-(ß1,2)-Man (tri-/tetraantennary complex oligosaccharide)
Griffonia simplicifolia I	GSA IB	terminal α-Gal
Viscum album	VAA	terminal α-Gal = terminal ß-Gal
Arachis hypogaea	PNA	terminal Gal-(ß1,3)-GalNAc
Artocarpus integrigolia	Jacalin	terminal Gal-(ß1,3)-GalNAc
Saphora japonica	SJA	terminal Gal-(ß1,3)-GalNAc > terminal Gal-(ß1,3)-GlcNAc
Griffonia simplicifolia I	GSA IA	terminal α-GalNAc > terminal α-Gal
Glycine max	SBA	terminal α-GalNAc > ßGalNAc > α-/ß-Gal
Dolichos biflorus	DBA	terminal GalNAc-α(1,3)-GalNAc >> α-GalNAc
Vicia villosa	VVA	terminal GalNAc-α(1,3)-Gal = GalNAc-α(1,6)-Gal = GalNAc-serine
Lotus tetragonolobus	LTA	terminal α-L-Fuc
Ulex europaeus I	UEA I	terminal α-L-Fuc

Fuc: fucose; Gal: galactose; GalNAc: N-acetylgalactosamine; Glc: glucose; GlcNAc: N-acetylglucosamine; Man: mannose; Neu5Ac: N-acetylneuraminic acid; sWGA: succinylated WGA.

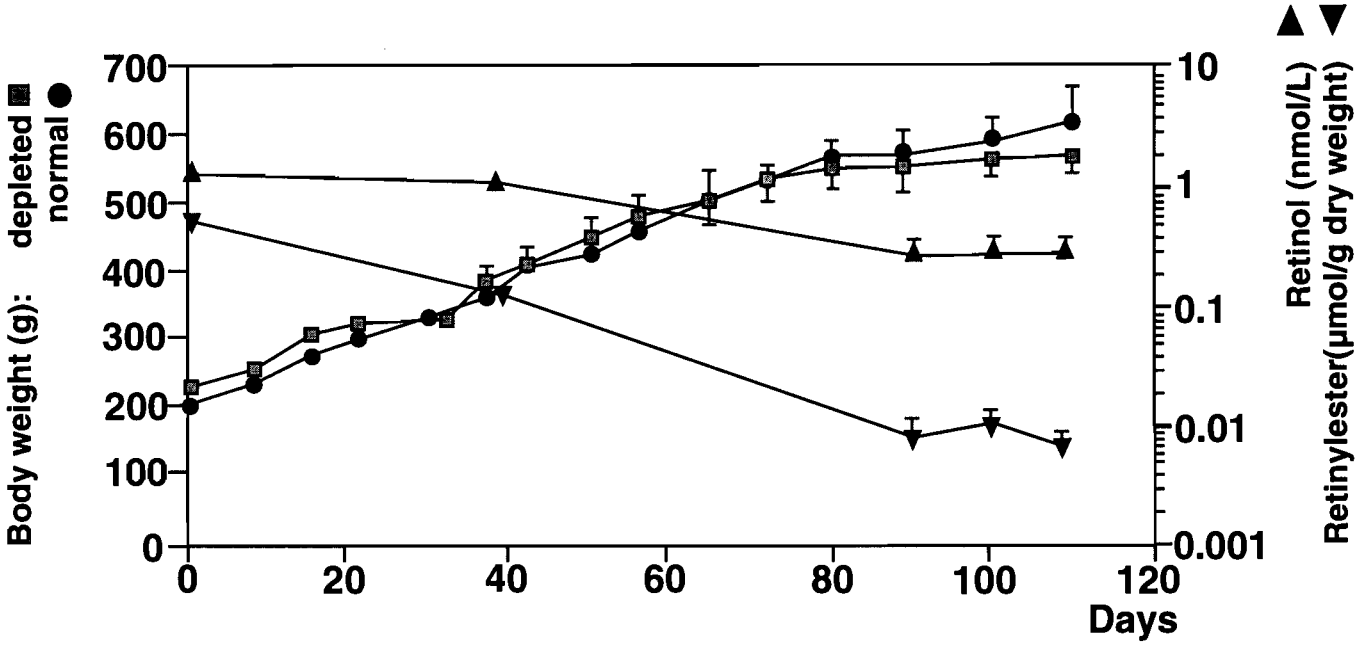


Fig. 1. Growth development and vitamin A status during the feeding of the vitamin A-free diet. Legend on the left side: Body weight (g) of depleted (○) and of control guinea pigs (●). Legend on the right side: Serum retinol (Δ: µmol/l) and liver retinyl ester (▲: µmol/g dry weight) concentrations from animals of the depletion group. The last values represent the situation at the morphological investigation stage.

deparaffinized with xylene, rinsed in two changes of absolute ethanol and gradually rehydrated. Additionally, specimens were snap-frozen in liquid nitrogen, cut at -20 ºC, and fixed in acetone. Tracheobronchial tissue of normally nourished rats (n=7) and mice (n=6) were treated similarly.

Conventional histochemistry

Serial sections were stained by HE and Azan. Presence of glycoconjugates was revealed by the periodic acid-Schiff (PAS) method. Acidic mucopolysaccharides were detected with Alcian blue 8GX at pH 1 or at pH 2.5 (Cook, 1990) and the Alcian blue technique involving the critical electrolyte concentration (Scott and Dorling, 1965).

Lectin-binding studies

To demonstrate lectin binding-patterns, the avidin-biotin-peroxidase (ABC) technique (ABC-Vectastain, Wiesbaden, FRG) was applied at room temperature (Hsu et al., 1981). Endogenous peroxidase-activity was suppressed with 0.3% H_2O_2 in methanol for 30 min. To reduce non-specific binding reactions by protein-protein interactions and binding of kit reagents to endogenous biotin, sections were treated for 15 min with 0.1% bovine serum albumin, with a solution of avidin, and with a solution of biotin in phosphate buffer (PBS), respectively. Then specimens were washed three times in PBS. Incubation with biotinylated lectins (Table 1; Camon, Wiesbaden, FRG) was performed for 45 min in a humidified chamber. The slides were then rinsed three times with PBS. Sections were incubated with the ABC-complex for 60 min and again washed in PBS. The colour reaction was developed with a fresh solution of 0.02% hydrogen peroxide and 0.1% diaminobenzidine-hydrochloride. The sections were rinsed in tap water, followed by distilled water. Finally, the samples were dehydrated, cleared and mounted. The intensity of lectin binding was graded subjectively and scored as negative= -, faintly visible= (+), weak= +, moderate= ++, and strong= +++. To eliminate terminal sialic acid molecules in some sections, slides were treated for 10 h with neuraminidase (Vibrio cholerae, 1 U/ml; Boehringer Mannheim, FRG) in 0.1 M sodium acetate buffer (pH 5.5; 0.04 M $CaCl_2$) at 37 ºC (Stoward et al., 1980).

Controls included (1) omission of the biotinylated lectins, and (2) pre-incubation of the probe in the presence of its appropriate glycoinhibitor.

Results

Laboratory data

Neither group revealed significant differences in growth development until day 100. The mean body weight of control animals killed on day 104 was 591±42 g, and in vitamin A-depleted guinea pigs 568±39 g. The retinol concentration in deficient animals was 1.22±0.23 nmol/ml serum, and in controls 3.53±0.45 nmol/ml serum. The retinyl ester stores of the liver amounted to 6.5±0.2 nmol/g dry weight in depleted animals and 563±45 nmol/g in normal fed guinea pigs. These data, together with the growth retardation, are indicative of the beginning of vitamin A deficiency. Neither alteration of the cornea nor respiratory infection occurred.

Morphology

Conventional histochemistry

Using conventional staining methods, three cell types, besides the secretory cells of bronchial glands, could be distinguished: ciliated columnar, basal, and goblet cells. We found no significant alterations in the number and morphology of ciliated cells. In contrast, the number of goblet cells was constantly decreased in hypovitaminosis A. Moreover, a considerable reduction of secretory granules was observed. In both groups, a large percentage of ciliated cells revealed no binding reaction or only a very faint marking by PAS. The periciliary layer of ciliated cells weakly labelled due to the presence of acidic molecules. The secretory granules of all mucin-producing goblet cells were PAS- and Alcian blue (pH 1.0; pH 2.5)-positive, indicating that granules of the secretory cells contained neutral, sulphated as well as carboxylated carbohydrates. In both groups, Alcian blue staining and PAS-reaction of the epithelium, respectively, showed an identical distribution. In depleted animals binding intensity was constantly diminished. The distribution of lectin-reactive profiles followed the pattern of PAS-positive sites. However, binding intensities showed no significant correlation. Glycoconjugate histochemistry was also investigated in the lung tissue of normal fed rat and mice. Results were essentially identical to the control group of guinea pigs.

Lectin-binding studies

Staining patterns were not significantly modified by the fixation procedures applied, even though the extent of color signal varied slightly, which was apparently dependent on the binding site and the particular lectin selected. Formalin proved to be an adequate fixative. However, Bouin-fixed specimens tended to display somewhat clearer pictures. In accordance with the literature, our results revealed no significant differences in the lectin-binding properties between different parts of the upper tracheobronchial tree (Geleff et al., 1986). In the distal segments of the airways quantitative variations were more notable, even in the same experimental group, although no qualitative differences were detected.

In guinea pigs (Table 2) incubation with the lectins DBA, LTA and succinylated WGA led to no staining reaction in any section investigated. Similarly, epithelial cells were negative, when GSA II and PNA were

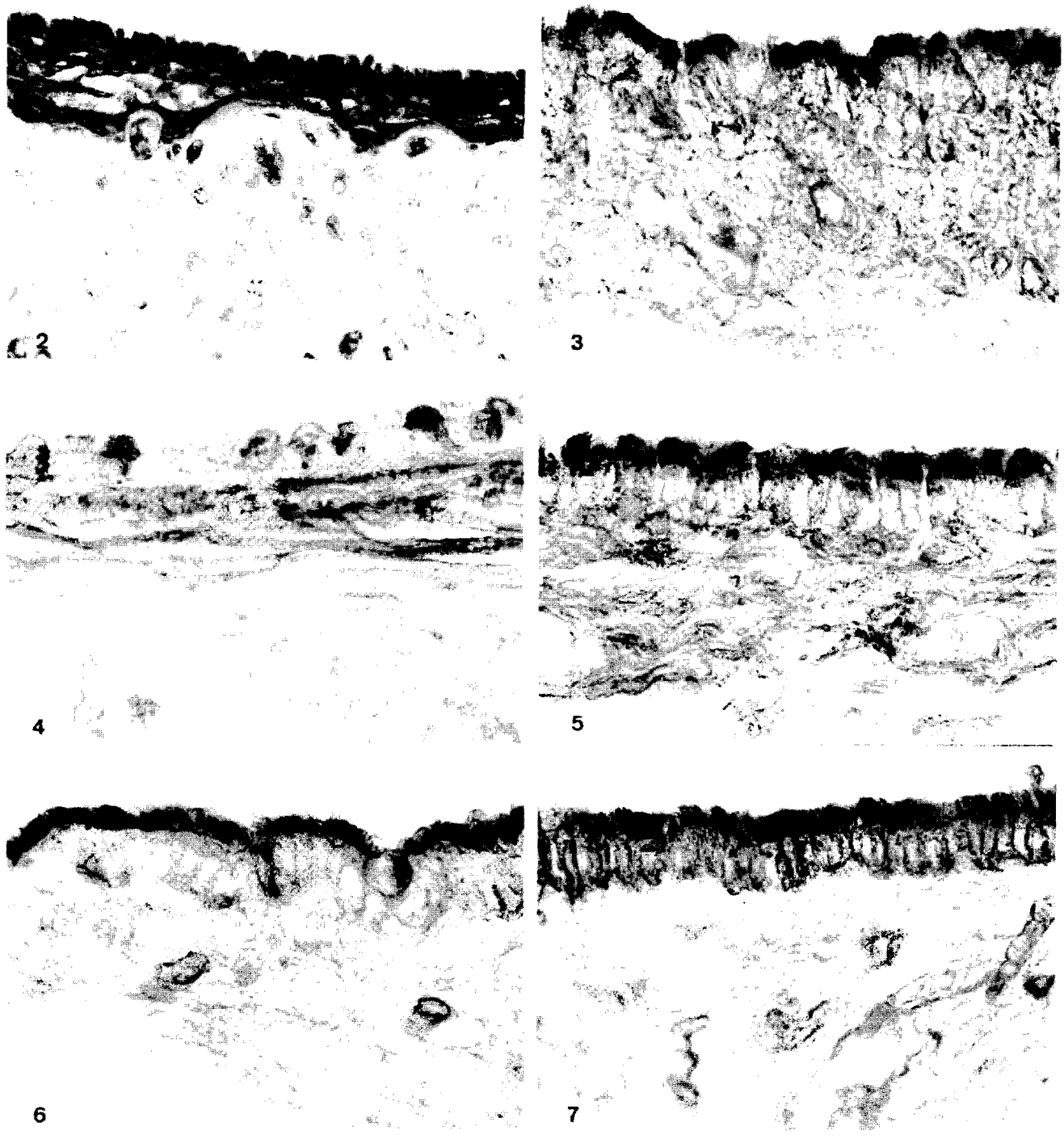


Fig. 2. Ubiquitous labelling by WGA in control animals. Trachea, paraffin section. x 110

Fig. 3. Intense LEA-labelling of ciliary processes in control animals. Trachea, paraffin section. x 420

Fig. 4. Binding patterns of PSA in control animals. Only goblet cells are intensely labelled. Trachea, paraffin section. x 480

Fig. 5. In hypovitaminosis A ciliary processes gain additional PSA-positivity. Trachea, paraffin section. x 480

Fig. 6. Faint cytoplasmic labelling by PHA-L in control specimens. Trachea, paraffin section. x 480

Fig. 7. In vitamin A-deficient animals cytoplasm gains binding affinity for PHA-L. Trachea, paraffin section. x 480

Table 2. Lectin staining patterns of tracheobronchial tissue (1). No differences were detected between depleted animals and control specimens. The only exception was the enhancement of connective tissue fibers in hypovitaminosis A after incubation with GSA II. (2) Labelling restricted to the basal parts of kinocilia; (3) uniform staining of cilia.

	CONTROL ANIMALS							VITAMIN A-DEPLETED ANIMALS			
	Ciliary cells		Secretory cells		Cartilage[1]		Connective tissue[1]	Ciliary cells		Secretory cells	
	Cytoplasm	Cilia	Cytoplasm	Granules	Chondrocytes	Matrix		Cytoplasm	Cilia	Cytoplasm	Granules
Con A	+++	++	+++	(+)	+	-	+++	+++	++	++	+
LCA	++	-	(+)	+	-	-	++	+	-	(+)	(+)
PSA	(+)	(+)	+	+++	-	-	++	+	+++	+	+++
LEA	++	+++	++	++	+	-	++	++	+++	++	++
STA	++	+++	+	++	+	(+)	++	++	++	+	++
DSA	++	++	++	++	(+)	-	+	++	+++	++	++
GSA II	-	-	-	-	-	-	+/+++[1]	-	-	-	-
WGA	+++	+++	+++	+++	+	(+)	+++	+++	+++	+++	+++
sWGA	-	-	-	-	-	-	-	-	-	-	-
ECA	-	-	-	++	-	-	-	-	-	-	++
RCA	++	+++	++	+++	-	-	+	++	+++	++	+++
PHA-E	+++	+++	+++	+++	-	-	+++	+++	+++	+++	+++
PHA-L	(+)	+++	(+)	+	-	-	(+)	++	+++	++	++
GSA I_B	-	+[2]	-	-	-	-	-	-	+[3]	-	-
VAA	++	+++	(+)	(+)	-	-	-	+	+	(+)	(+)
PNA	-	-	-	-	++	-	-	-	-	-	-
Jacalin	++	+++	+	+	++	-	++	+	++	+	+
SJA	-	-	-	++	-	-	-	-	-	-	++
GSA I_A	(+)	(+)	+	+	-	-	-	(+)	(+)	+	+
SBA	(+)	(+)	+	+	-	-	(+)	(+)	(+)	+	+
DBA	-	-	-	-	-	-	-	-	-	-	-
VVA	++	++	+	(+)	-	-	-	-	-	-	-
LTA	-	-	-	-	-	-	-	-	-	-	-
UEA I	-	-	-	-	-	-	-	-	-	-	-

applied. After enzymatic release of terminal sialic acid moieties from glycoconjugates by neuraminidase treatment, ciliary cells and loose connective tissue gained additional PNA-reactivity in both groups. However, in ciliary cells this effect was significantly less impressive in retinol-deficient animals.

Ciliary cells. DSA, GSA IA, PHA-E, SBA, and WGA (Fig. 2) homogeneously labelled ciliary cells, with the exception of nuclei. Compared to the remaining cytoplasm, the quantity of accessible ligands on cilia for Con A was somewhat reduced, whereas jacalin, LEA (Fig. 3), PHA-L, RCA, and STA showed preference for these structures. Staining of LCA was confined to apical (secretory?) granules. In control specimens GSA I_B-positivity was restricted to the basal part of kinozilia. In aggregate, most lectins demonstrated identical distribution patterns, irrespective of the vitamin A status. However, epithelial binding intensity of jacalin, LCA, STA and VAA was constantly reduced in depleted guinea pigs. In contrast, PSA-labelling of kinozilia was dramatically enhanced (Figs. 4, 5), as was the binding affinity of the cytoplasm for PHA-L (Figs. 6, 7). In deficient animals, the apical parts of the kinozilia were additionally labelled by GSA I_B (Figs. 8, 9). Staining of epithelial cells by VVA was restricted to control specimens.

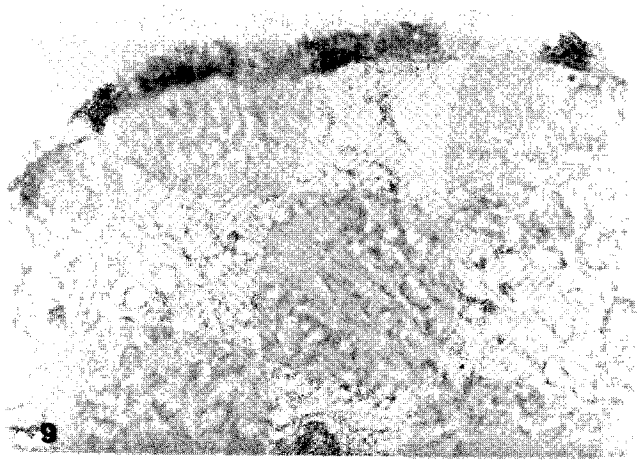
Secretory cells. Goblet cells were specifically labelled by ECA (Fig. 10) and SJA. Additionally, PSA-staining was characteristically enhanced when compared to ciliated cells. The remaining lectins demonstrated an identical or reduced binding intensity. No differences were observed between either group.

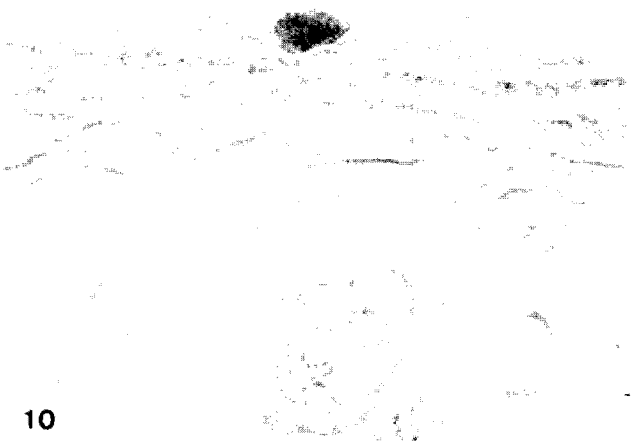
Basal cells. In both cohorts no significant difference could be demonstrated when compared to the cytoplasm of ciliary cells.

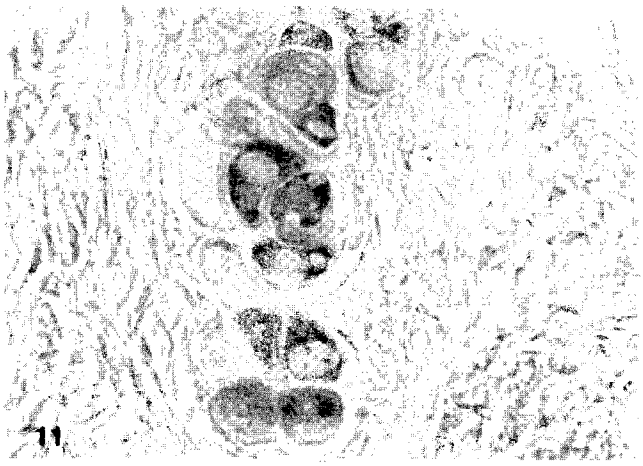
Cartilage. STA and WGA showed a faint binding intensity for the extracellular matrix. All other lectins were constantly negative. The cytoplasm of chondrocytes revealed a granulated binding pattern after incubation with Con A, LEA, PNA (Fig. 11), STA, or WGA, respectively. DSA and jacalin revealed a rather even distribution. Results were identical in both groups.

Connective tissue. In vitamin A-depleted guinea pigs binding affinity of GSA II was significantly enhanced when compared to control animals (Figs. 12, 13). The remaining lectins demonstrated no characteristic differences between either group. Binding intensity of PHA-E was especially strong.

Species differences. With the exception of DBA, PNA, RCA, UEA I, and VVA, most lectins demonstrated a nearly identical binding pattern in the 3 species investigated (Table 3). In rats, GSA I_B was a specific







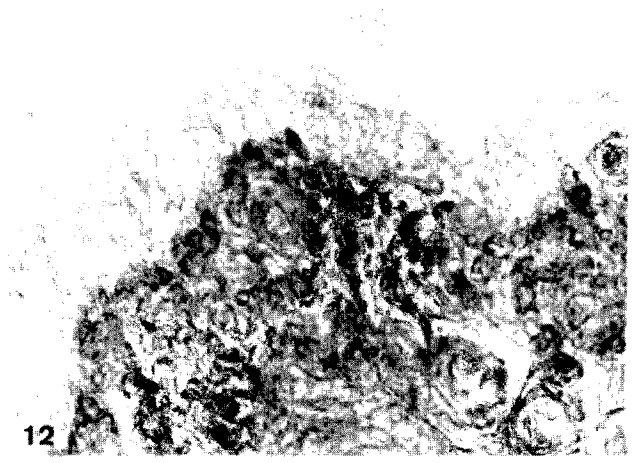


Fig. 8. Restricted affinity for GSA I_B in normally nourished animals. Trachea, paraffin section. x 240

Fig. 9. Positivity of ciliary processes in depleted animals. GSA I_B. Trachea, paraffin section. x 240

Fig. 10. Positive reactivity of goblet cells for secretory granules of goblet cells. Hypovitaminosis A. ECA. Trachea, paraffin section. x 420

Fig. 11. Granulated binding patterns of PNA in the cytoplasm of chondrocytes. Bronchus. paraffin section. x 480

Fig. 12. In control specimens connective tissue fibres show intermediate affinity for GSA II. Trachea, paraffin section. x 480

Fig. 13. Strong positive binding reactivity of GSA II lectin to connective tissue fibres is evident in depleted animals. Trachea. x 480

Table 3. Species differences of the lectin affinity incontrols animals: cytoplasm/cilia of ciliary cells.

	DBA	PNA	RCA	UEA I	VVA
Guinea pigs	-/-	-/-	++/+++	-/-	++/++
Rats	+	-/-	(+)/(+)	-/-[1]	-/-
Mice	-/-	++/++	+/++	-/(+)	-/-

1: in rats, UEA I was a specific marker of secretory.

marker of basal cells, whereas cilia were negative.

Control reactions. When biotinylated lectins had been omitted, the slides exhibited no label deposition. After pre-incubation of lectins with their complementary sugars, no staining was detected, with the exception of DSA and PHA-E, where a weak labelling reaction was constantly observed. No binding occurred in any of the control reactions in the presence of glycoinhibitors, excluding protein-protein interactions as a reason for labelling.

Discussion

Marginal vitamin A deficiency is characterized by nearly exhausted liver vitamin A stores and moderately decreased retinol plasma levels without any typical clinical signs of depletion such as Bitot spots or xerophthalmia. Numerous epidemiological studies have demonstrated that the morbidity of respiratory tract infections is significantly increased during subclinical vitamin A deficiency (Arthur et al., 1992). This indicates that even when retinol plasma levels are in a low but normal range epithelial functions seem to be severely affected (Olson, 1986). In contrast, conventional morphological analysis fails to demonstrate any characteristic structural defect of the respiratory epithelium. In a first step to analyze this problem further, the distribution of different cell populations was quantified in serial sections in pair-fed animals. Because it was occasionally difficult to classify cell structures in 6-7 µm paraffin sections, our observations were confirmed by morphometric investigations of 0.5 µm semithin sections of tracheal tissue, as described in a previous publication (Stoff et al., 1992). Quantification demonstrated a small but constant reduction of goblet cells in depleted animals showing 16.07 (±1.45) secretory cells in depleted guinea pigs, compared to 19.01 (±1.51) cells in pair-fed animals (t-test 3.89; significance level: 0.05). The number of ciliated cells was almost unchanged with 21.92 (±2.56) ciliated cells/200 µm epithelium in hypovitaminosis A, compared to 21.76 (±0.94) cells in control sections (t-test: 0.14). These results suggest that cellular regeneration of bronchial ciliated cells is not significantly affected in early deficiency. In contrast, the findings are in agreement with the hypothesis that glycoconjugate metabolism of the respiratory system seems to be modified by vitamin A status at a point in time when morphological investigations reveal no or only uncertain alterations. Similar observations have been described in other organ systems such as epidermis and testes (Elias et al., 1983).

Alcian blue is a useful histochemical indicator of presence of negatively-charged proteoglycans (Spicer, 1960). The PAS reaction specifically detects accumulations of neutral carbohydrates (Mowry, 1963). A loss of PAS-reactivity has been reported in vitamin A-deficient hamster tracheal organ cultures (Chopra and Joiakim, 1991) thus corroborating our observations. Analogous results were observed in the cloned fetal epithelial cell line M3E3/C3 where production of PAS- and alcian blue-positive granules were critically dependent on the vitamin A level (Emura et al., 1988). Because of the complex, often branched structure, interfering with Schiff reactivity, lectin-binding oligosaccharides are generally not or only very faintly positive. This may explain the observation that no significant correlations were found between lectin-binding patterns and the results of dye-employing histochemistry. Since no dramatic effects should be assumed, it is noteworthy that from the battery of applied lectins a minority indeed revealed characteristic modifications in the staining patterns during early retinol deficiency. This correlates with biochemical studies demonstrating that not all, but distinct glycosyl-transferases can show retinoid-dependent changes in their activities (Gudas et al., 1994).

Correlation of the binding characteristics of the lectins Con A, LCA and PSA in normal and depleted animals indicates a significant induction of the (α1,6)-fucosyltransferase activity in ciliary membranes already in marginal vitamin A deficiency, whereas in the remaining respiratory tract no differences were detected. In contrast to our observations a vitamin A dependency of fucosyl-addition to glycoproteins in respiratory epithelium of rat and hamster have been reported in biochemical in vitro-investigations (Bonanni and de Luca, 1974). Additionally, expression of branching enzymes seems to be modified by the retinoid status. This conclusion can be drawn from the staining results of PHA-E and PHA-L, respectively. Both molecules show affinity only for galactose-(ß1,4)-N-acetyl-glucosamine sequences of oligosaccharides that are branched at the vicinal mannose group. The results of this study demonstrate that biantennary molecule chains detected by PHA-E are not modified, whereas production of tri- or tetraantennary glycoconjugates labelled by PHA-L (Cummings and Kornfeld, 1982) is significantly enhanced in the cytoplasm of depleted animals.

No significant differences were detected between either group, using DSA, LEA and STA staining. This result indicates that expression of the sequence N-acetylglucosamine-(ß1,4)-N-acetylglucosamine that is present in the core of N-linked sugar chains is not significantly modulated by vitamin A. In contrast to the N-acetylglucosamine-specific sWGA that was negative,

native WGA also exhibits strong affinity to sialic acid in the terminal position (Maget-Dana et al., 1981). Its binding pattern therefore indicates an ubiquitous distribution of this carbohydrate in epithelial as well as mesenchymal tissues that evidently is not significantly altered by the vitamin A status. The markedly reduced binding affinity of VAA for epithelial cells in depleted guinea pigs is indicative of a reduced galactosyltransferase activity. Analogous results have been documented by an galactosyltransferase assay (Plotkin and Wolf, 1980; Cummings and Mattox, 1988).

Presence of PNA-positive ciliary cells following neuraminidase treatment gives evidence for the sialylation of terminal galactose-(ß1,3)-N-acetylgalactosamine sequences with sialic acid molecules (Hennigar et al., 1987). In various species it has been shown that sialyltransferase activity is not expressed in bronchotracheal epithelium until postnatal development (Honda et al., 1989). Abolition of sialyltransferase activity is regarded as a sensitive marker of altered cell metabolism in inflammation (Cooper et al., 1987) or tumor development (Lehmann et al., 1984). Our results indicate that enzyme activity is not abolished in sustained marginal hypovitaminosis A. Analogous lectin-binding results have been reported in hamster tracheal explants (Chopra and Joiakim, 1991).

The histochemical staining patterns of jacalin concur with the assumption of masking of terminal galactose by sialic acid. The functional importance of the reduced labelling in depleted animals remains to be determined. Although GSA I_B also exhibits selective affinity for terminal galactose its binds to this carbohydrate only when it is linked in an α-position (Peters and Goldstein, 1979). We assume that ciliar positivity in depleted animals can be explained as the result of reduced glycosyltransferase activity. As a consequence, sugar molecules, normally located within the carbohydrate chain, that will remain in the terminal position (Kobata and Takasaki, 1991), serve as a lectin receptor. Alterations in the composition of oligosaccharide structures may contribute to the enhanced infect susceptibility in reduced retinol homeostasis. Bacteria have been found to express numerous lectins that can specifically interact with complex carbohydrate structures on mucosal surfaces (Weir, 1988). A low concentration of these glycoconjugates on cell surfaces may prevent adherence of microbes on tracheal epithelium. It was demonstrated that modification of mucosal oligosaccharide composition can change the delicate host-parasite interaction (Ofek and Sharon, 1990). However, dysfunctional immune functions may also be responsible for decreased resistance to infections in marginal vitamin A deficiency (Ross and Haemmerling, 1994).

It is noteworthy that Chopra and Joiakim (1991) reported induction of DBA-affinity by vitamin A deficiency in tracheal cultures of hamsters. It is difficult to relate these findings to our results because several variables, such as species differences and cell culture conditions, may account for this disparity. The reasoning is supported by literature data that identical binding reactions of DBA, GSA I_A, SBA, or VVA, respectively, should not be expected when these parameters are applied (Spicer and Schulte, 1992). Contrary to our negative results, «a clear to very strong staining» was reported by Geleff and coworkers (1986) in tracheal epithelium of guinea pigs after incubation with UEA I. This discrepancy is difficult to explain. Possible subspecies-dependent discrepancies in the glycoconjugate expression will also have to be rigorously excluded (Schulte and Spicer, 1983). However, the negative binding reaction of LTA, confirmed in both studies, is a strong argument that terminal α-fucosyl residues are apparently not expressed (Pereira et al., 1978; Alroy et al., 1988).

Taken together, our results indicate that the beginning morphological transformation in hypovitaminosis A, at least phenomenologically, correlates with modified expression of oligosaccharide structures. It is reasonable to assume that the modifications detected in this study are not pathognomonic markers. They may instead reflect the profound and varied influences of retinoid status on cell physiology. In interpreting the results, species-dependent differences in the expression of terminal glycoconjugates also have to be considered. However, altered lectin-binding patterns are apparently not only a sensitive tool to reveal perturbed glycoconjugate metabolism at an early date in vitamin A deficiency. As shown in comparative tumor pathology, transient alterations of cell surface carbohydrates may play an important role in cell interactions (i.e. metastatic behaviour; Couch et al., 1988). In further studies, the primary mechanisms of retinoid action remain to be exactly addressed. It is not known whether retinoic acid-receptor complex interactions directly induce the synthesis of oligosaccharide processing enzymes or cause a cascade of factors which regulate enzyme expression. Besides its direct effects on the post-translational glycosylation level, vitamin A is additionally involved in the synthesis of multiple protein cores of mucins or proteoglycans (e.g. mucus core proteins; Manna et al., 1994). It has been previously demonstrated that the synthesis of some acceptor proteins for glycosylation can be significantly down-regulated or even interrupted after squamous differentiation of tracheal epithelial cells. This may additionally result in significant alterations of the lectin-binding patterns. Therefore, to study early morphological effects of hypovitaminosis A in vivo, the investigation of glycoconjugate expression seems to be especially suitable. Likewise, retinoids are known to modulate expression of endogenous receptors for glycoconjugate structures in vitro (Lotan et al., 1989; Gabius et al., 1990). When the glycoepitopes are not merely considered as passive structural elements, but also as information-bearing ligands for endogenous lectins (Gabius, 1991; Gabius and Gabius, 1993), this aspect deserves similar attention in further lines of

investigation.

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