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Light-microscopic examination of ABH and Lewis antigens in human tracheal and epiglottic glands using the Avidin-Biotin-Peroxidase Complex technique

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Summary. The localization of ABH and Lewis antigens was examined in formalin-fixed, paraffin-embedded human tracheal and epiglottic glands using monoclonal anti A, B, H, Le^a and Le^b antibodies. The mucous cells of the glands showed reactivity with antibodies corresponding to the respective ABO blood groups of the tissue donors.

The mucous cells from one blood group A, Le(a−b−) individual showed no reactivity with any antibodies and those from another blood group A, Le(a−b−) individual showed reactivity only with anti A antibody. In individuals from blood group Le(a+b−) of all ABO groups, the mucous cells reacted exclusively with anti Le^a. In blood group O, Le(a−b+) individuals, the mucous cells showed intense reaction with anti H and Le^b antibodies and weak to moderate reactivity with anti Le^a. In Le(a−b+) individuals of A_1, B and A_1B blood groups, the mucous cells showed strong reactivity with anti A and/or B antibodies, moderate with anti Le^b, weak or no activity with anti Le^a and absent with anti H. In blood group A_2 Le(a−b+) individuals, the mucous cells stained with anti A were weakly stained or completely unstained with anti H antibody, but cells negative with anti A gave strong positive reactions with anti H antibody.

Key words: ABH antigens, identification from tracheal and epiglottic glands – Lewis antigens, in tracheal and epiglottic glands – AB complex

Zusammenfassung. Mit Hilfe monoklonaler Antikörper wurden die ABH- und Lewis-Antigene in Drüsen von Trachea und Epiglottis in formalinfixiertem und paraffineingebettetem Material untersucht. Die Reaktion der mukösen Drüsenzellen zeigte eine Übereinstimmung mit der festgestellten ABO-Blutgruppe. Bei einer Person der Blutgruppe A, Le(a−b−) war keine

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Reaktion mit Antikörpern nachweisbar, bei einer anderen der gleichen Blutgruppe nur eine Reaktion mit Anti-A. Die mukösen Zellen aller Untersuchten der Blutgruppe Le(a+b−) reagierten nur mit Anti-Le^a. Bei Vorliegen der Blutgruppe O Le(a−b+) ergab sich eine sehr intensive Reaktion mit Anti-H und Le^b und schwache bis mittlere Reaktionen mit anti Le^a. Bei Vorliegen der Blutgruppe Le(a−b+) und A_1, B und A_1B zeigten die mukösen Zellen eine starke Reaktion mit Anti-A und/oder Anti-B, eine mittlere Reaktion mit Anti-Le^b, eine schwache oder keine Reaktion mit Anti-Le^a und keine mit Anti-H. Bei Vorliegen der Blutgruppe A_2, Le(a−b+) zeigten die Zellen, die mit Anti-A reagierten, keine Reaktion mit Anti-H, und die, welche negativ mit Anti-A waren, eine positive Reaktion mit Anti-H.

Schlüsselwörter: ABH-Antigene, in Tracheal- und Epiglottisdrüsen – Lewis-Antigene, in Tracheal- und Epiglottisdrüsen – Avidin-Biotin (AB)-Komplex

Introduction

ABH und Lewis blood group substances on human erythrocytes are also found in secretions from several types of cells and tissues. The distribution of blood group substances in tissue sections has been investigated with fluorescent antibodies (Szulman 1960; Szulman and Markus 1973) and a red cell absorption method (Ishiyama 1979). Recent light-microscopic research with lectin-histochemical and peroxidase-antiperoxidase (PAP) methods showed that A, B and H antigens can also be located in kidney, saliva glands, pancreas and Hassall's corpuscles of the thymus (Pedal and Baedecker 1985; Ito et al. 1985, 1986; Mizumoto 1986). Tracheo-bronchial epithelium in man has also been investigated using lectin-histochemical, PAP and biochemical methods for the detection of complex carbohydrates and blood group antigens (Boat and Cheng 1980; Schulte and Spicer 1983; Pedal and Baedecker 1986). The known and proposed structures of oligosaccharides in human tracheo-bronchial glycoproteins include α-N-acetyl galactosamine-threonine (serine), N-acetylneuraminic acid-(2→3)-β-galactose-(1→3)-N-acetylgalactosamine-threonine (serine) (Boat and Cheng 1980). Results obtained using lectin-histochemical and biochemical methods have also been subject to criticism, since these methods have not answered important questions, such as which cells produce blood group substances and whether lectins really recognize the blood group substances.

In the present study, monoclonal anti A, B, H, Le^a and Le^b antibodies were used to detect these blood group substances in human tracheal and epiglottic glands and the biosynthetic process of the blood group antigens in tracheal and epiglottic glands is discussed with reference to these findings.

Materials and methods

Specimens of human trachea and epiglottis used in this study were collected from a total of 88 autopsies. Blood group typing for ABO and Lewis system of the tissue donors was performed by the routine hemagglutination test. The distribution of the blood groups of the specimens is listed in Table 1.

Table 1. Distribution of ABO and Lewis blood groups of specimens tested in this study

	a−b+	a+b−	a−b−	a+b+	Total
A_1	22	6	2	0	30
A_2	6	2	0	1	9
B	6	1	0	0	7
O	25	7	1	1	34
AB	5	1	2	0	8
	64	18	5	2	88

Tissue specimens were fixed in 10% neutralized formalin, embedded in paraffin and sectioned at 6 μm. The tissue sections were deparaffinized, washed with absolute and 96% ethanol and immersed in 0.3% H_2O_2/methanol solution for 30 min to inactivate endogenous peroxidase. The sections were rinsed in Tris-HCl buffer containing NaCl (0.9%) and bovine serum albumin (1 mg/ml) for 30 min. Tissue sections were then incubated with mouse monoclonal A, B, H, Le^a and Le^b antisera diluted with Tris-HCl/NaCl buffer for 30 min. Antibody dilutions varied from batch to batch, but were previously assessed by checkerboard titration. Monoclonal anti A and B antibodies (Biotest, West Germany) and anti Le^a and Le^b antibodies (Immucor, West Germany; Biotest, West Germany; Fresenius, West Germany) were employed and in several specimens the reactivity with Ulex europaeus agglutinin I was also examined. The slides were gently rinsed in Tris-HCl buffer for 10 min and incubated with biotinylated rabbit anti-mouse immunoglobulins (Dakopatts, Denmark) for 30 min. After rinsing in Tris-HCl buffer, the slides were immersed in 0.1 *M* acetate buffer, pH 5.2, containing 3-amino-9-ethylcarbazole (0.3 mg/ml), N.N. dimethyl-formamide (0.07 mg/ml) and hydrogen peroxide (1 μl/ml) for 5 min. After the final wash with Tris-HCl buffer, the slides were counterstained with hematoxylin. All the steps were carried out at room temperature.

Results

The negative control sections showed no reaction apart from the background staining of hematoxylin. Blood group-positive reaction was observed in the mucous cells of the glands, epithelial cells, goblet cells, internal material of ducts in the gland, duct system, endothelial cells of the blood vessels and erythrocytes in the trachea and epiglottis. No positive reactions with ABH and Lewis antibodies were recognized in the serous cells of the glands and cartilage tissue.

In every sample the mucous cells of the glands, the epithelial cells and the goblet cells reacted uniformly. Therefore, to simplify the text only the mucous cells are referred to. The mucous cells from one of the blood group A_1 Le(a−b−) individuals showed no reactivity with any of the antibodies tested in this study. In this specimen, only erythrocytes and endothelial cells of blood vessels were stained with anti A and H antibodies. In the other individual of blood group A_1 Le(a−b−) the mucous cells were strongly stained by anti A antibody but were not stained by any other antibodies. In all individuals of blood group Le(a+b−) of all ABO groups examined in this study, the mucous cells showed exclusive reactivity with anti Le^a antibody. In blood group O Le(a−b+) individuals the mucous cells showed intensive reactivity with anti H

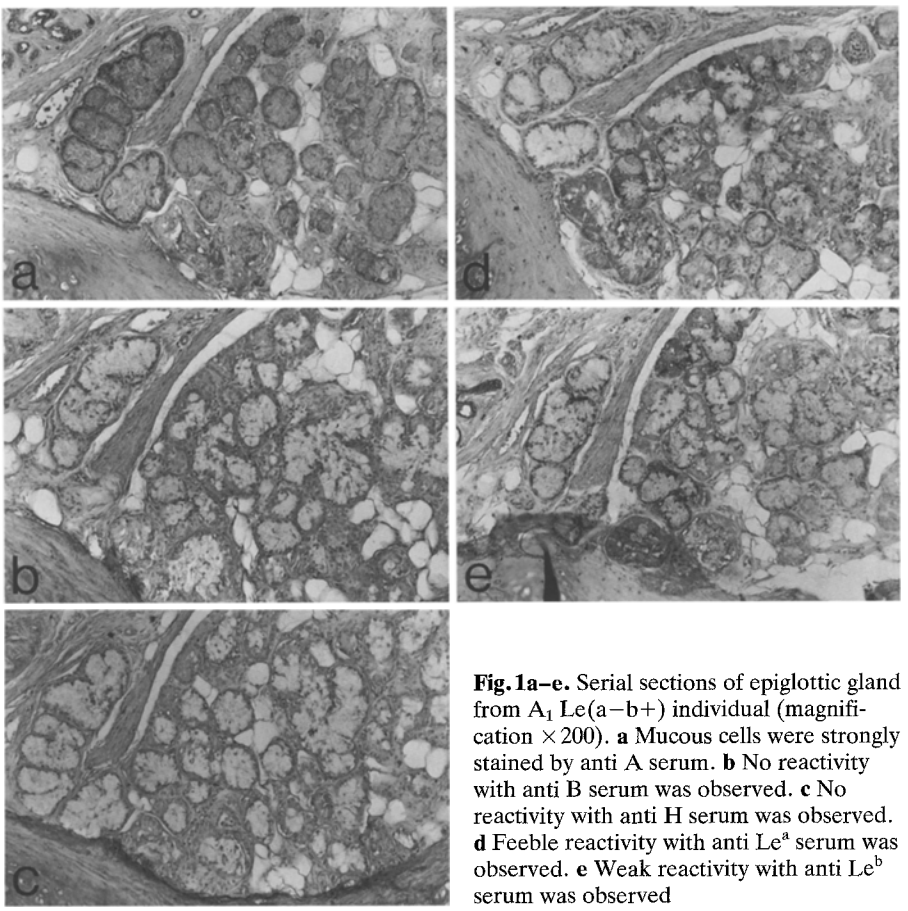

Fig. 1a–e. Serial sections of epiglottic gland from A_1 Le(a−b+) individual (magnification ×200). **a** Mucous cells were strongly stained by anti A serum. **b** No reactivity with anti B serum was observed. **c** No reactivity with anti H serum was observed. **d** Feeble reactivity with anti Le^a serum was observed. **e** Weak reactivity with anti Le^b serum was observed

antibody and with anti Le^b and a weak to moderate reaction with anti Le^a. The mucous cells from all A, B and AB Le(a−b+) individuals showed intensive specific staining with the corresponding anti A and/or B antibodies. The mucous cells showed weak reaction with anti Le^b, very weak or no reaction with anti Le^a and no reaction with anti H.

There was a definite difference in the reactivity with anti H antibody between A_1 and A_2 secretor individuals (Figs. 1, 2). Although the mucous cells of the tracheal and epiglottic glands from blood group A_1 individuals showed absolutely no reaction with anti H antibody, the mucous cells from A_2 individuals showed clear reactivity. In blood group A_2 individuals, the mucous cells stained with anti A antibody were weakly stained or completely unstained by anti H antibody, but negative cells with anti A antibody gave strong positive reactions with anti H antibody. On the other hand, Ulex europaeus agglutinin I showed intensive reactivity in the mucous cells from both blood group A_1 and A_2 individuals. Furthermore, the mucous cells from blood group A_2 Le(a−b+) individuals showed moderate reactivity with anti Le^b antibody and weak to moderate reactivity with Le^a antibody.

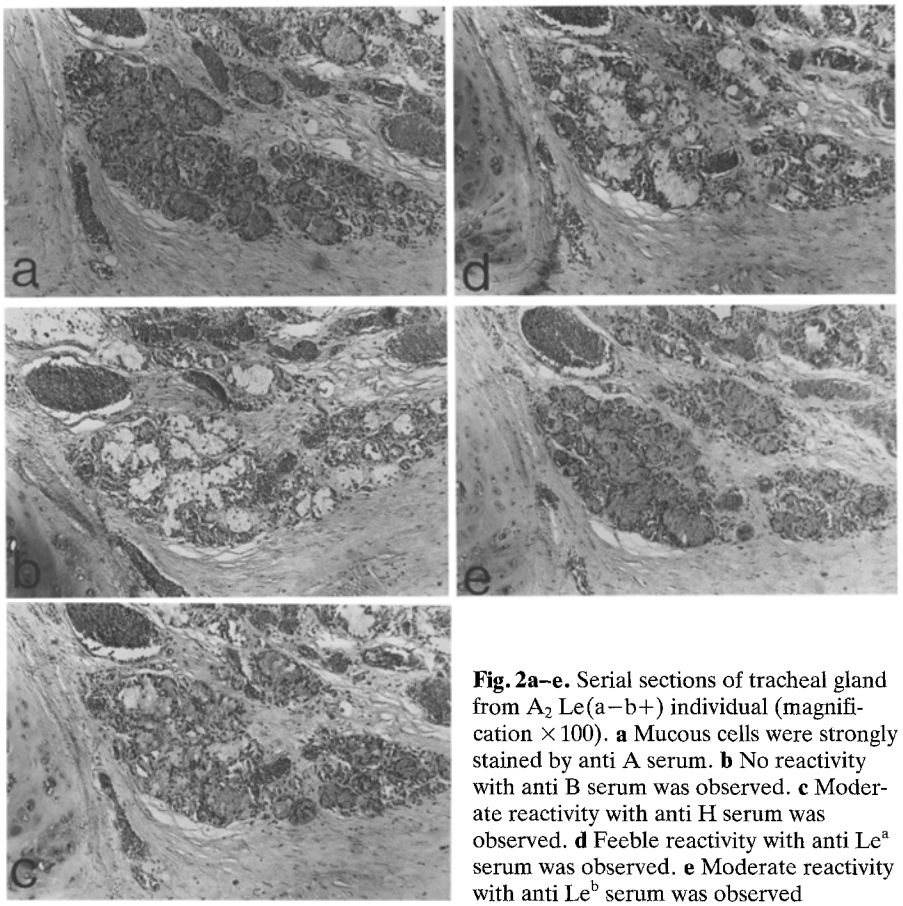

Fig. 2a–e. Serial sections of tracheal gland from A_2 Le(a−b+) individual (magnification ×100). **a** Mucous cells were strongly stained by anti A serum. **b** No reactivity with anti B serum was observed. **c** Moderate reactivity with anti H serum was observed. **d** Feeble reactivity with anti Le^a serum was observed. **e** Moderate reactivity with anti Le^b serum was observed

Discussion

In this study, it was found that the monoclonal antibodies reacted specifically with the mucous cells of the tracheal and epiglottic glands. It is well known that the ABH, Secretor (Se) and Lewis (Le) genes influence the quantity and quality of blood group antigen expression in the secretor tissues (Watkins 1980). The ABH antigens are glycoproteins and glycolipids, in which type 1 and type 2 carbohydrate chains carry the antigen determinations. Type 3 and type 4 chains have also recently been demonstrated in several tissues and organs (Hakomori 1984). Type 1 and type 2 chains co-exist in epithelial tissues and secretions (Watkins et al. 1981). The Lewis antigens are only synthesized on type 1 chains (Lemieux et al. 1981). According to the biosynthetic scheme of the ABH and Lewis antigens proposed by Watkins (1980), the antigens are composed of a genetically determined sequential addition of sugar units to the precursor substance by the glycosyltransferase specified by the Se, H, Le, A and B genes.

In the present study, type 2 chain-specific anti H antibody failed to detect the H antigen in O Le(a+b−) individuals. This would indicate that the Se gene

Table 2. Staining results of mucous cells in tracheal and epiglottic glands using avidin-biotin-complex technique and antibodies

Blood groups		Antibodies			
ABO	Le	A/B	H	Le^a	Le^b
A/B	a−b+	pos	neg	vw	w
A/B/H	a+b−	neg	neg	pos	neg
H	a−b+	neg	pos	wm	pos
A	a−b−	pos/neg	neg	neg	neg
AB/H	a−b−	neg	neg	neg	neg
		A	H	UEA-I	
A_1		pos	neg	pos	
A_2		pos	pos	pos	

pos: positive, neg: negative, vw: very weak, wm: weak to moderate

controls H antigen expression on both type 1 and 2 chains in trachea and epiglottic glands.

The results, summarized in Table 2, together with the biosynthetic pathway proposed by Watkins (1980) introduce a concept described below. In the mucous cells from Lewis-positive individuals the type 1 precursor substance may undergo conversion into either H or Le^a substance and H substance may be converted into A or B substances by the A or B gene-specific transferase, or into Le^b substance by the effect of the Lewis enzyme. The competition of the Lewis gene-product for a common acceptor molecule may occur at two points between H and Le enzymes and between A, B, and Le enzymes. The different staining intensity in the blood group A, Le(a−b+) or B, Le(a−b+) individuals with anti A or B and Le^b antibody is probably due to the amount of the antigens determined by the competition between the A or B and Le-glycosyl transferases and by the proportion of the type 2 chain to which the Le enzyme has no access. In non-secretors, in areas where there is no formation of H substance, Le enzyme is free from competition, so that the exclusive production of Le^a substance is expected. The weak reactivity with anti Le^a serum in the blood group Le^b individual could be due to the presence of small amounts of Le^a molecules not transformed into Le^b substance.

In this study an interesting reaction was found where the mucous cells from blood group A_1 secretor individuals reacted with anti A antibody, but the cells from A_2 individuals reacted with anti A and H antibody showing a mosaic-like pattern. Ulex europaeus agglutinin I stained the mucous cells from A_1 and A_2 to almost the same degree. Family studies have shown that A_1 and A_2 characters are inherited as alleles of the A_1, A_2, B, O system (Friedenreich and Zacho 1931). A_2 red cells react strongly with anti H reagents and weakly with anti A reagents and A_1 red cells show the reverse reaction with these reagents. Distinct enzymes of A_1 and A_2 have been identified by isoelectric focusing (Topping and

Watkins 1975) or by differences in their optimum pH activity (Cartron et al. 1976). The A_2 enzyme is 5–10 times less efficient than the A_1 enzyme with Lacto-N-fucopentanose as substrate (Schachter et al. 1971) and the two enzymes have different cation requirements (Schachter et al. 1973). From the results obtained in this study it is suggested that the A_2 gene is a "weak" gene so that in A_2 individuals the H specificity recognized by monoclonal anti H antibody attains maximum expression, but in individuals of group A_1 the enzyme determined by the "stronger" gene interferes with expression of the H specificity. These findings have a value in forensic medicine from several points of view: firstly, it is probably a question of a model-dependent reaction pattern which in this form, or a modification of this, is also relevant to other organs. The tissue-specific variations of the expression of ABH antigens described by Oriol (1988) must certainly be taken into consideration. In addition, these findings contribute to the ever increasing complications of the biosynthesis of the extensive ABH and Le antigen family. Finally, the findings allow the possibility to determine the previously unknown secretor status of a corpse.

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