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ABH-related antigens in human male genital tract

A histochemical examination

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Summary. The localization of ABH related antigens in human male reproductive tract was examined using monoclonal antibodies and an avidin biotin complex method. No positive reaction with blood group antibodies on spermatozoa was observed in testis and ductus epididymidis apart from erythrocytes and endothelial cells. The expression of ABH and ABH related antigens in ductuli efferentes testis, ductus epididymidis, seminal vesicle and prostate was complexly coded by a combination of H, Se, Le and X genes. The results obtained in this study indicate that the ABH antigens detected on spermatozoa of seminal stains are coating antigens and not inherent to the cell membrane, and the ABO, H, Se, Le and X genes are subjected to a tissue-dependent differential expression.

Key words: Immunohistochemistry – ABH-related antigens – Human male genital tract

Zusammenfassung. Die Lokalisation (and Verteilung) der Antigene des ABH-Komplexes im Bereich der inneren Geschlechtsorgane des Mannes wurde mittels monoklonaler Antikörper unter Benutzung einer Avidin-Biotin Technik untersucht. Dabei konnten positive Reaktionen im Hoden und im Ductus epididymidis lediglich an Erythrozyten und Endothelzellen beobachtet werden. Die Expression von ABH-Antigenen in den Ductuli efferentes testis, im Ductus epididymidis, in den Samenbläschen und der Prostata wird offensichtlich komplex durch H-, Se-, Le- und X-Gene kodiert. Die Resultate der vorliegenden Untersuchungen zeigen, daß die ABH-Antigene der Spermienoberfläche offensichtlich aus der Samenflüssigkeit stammen und die ABO-, H-, Se-, Le- und X-Gene gewebsabhängig unterschiedlich exprimiert werden.

Schlüsselwörter: Immunohistochemistry – Antigene des ABH-Komplexes – Männlicher Genitaltrakt

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Introduction

In the forensic practice, various kinds of materials collected from scenes of crime are examined for the identification of the perpetrator. Since the identification is done mainly by detection of blood groups in biological materials, it is indispensable to establish the detection methods for blood group antigens in these forensic materials. Although it is widely accepted that A, B, H antigens can be demonstrated in body fluids by absorption-inhibition methods, these have several drawbacks because the different soluble blood group antigens cannot be separated in a mixture. If the cellular antigens could be attributed to a constitutent of a mixture of body fluids and/or bacteria, this would prove to be of great assistance in the interpretation of the results. We reported a detection method for ABH antigens from the buccal cells in saliva and from the spermatozoa in seminal fluid (Brinkmann et al. 1986). Although the determination of the ABH group on spermatozoa is now generally accepted, it has been a controversial subject whether the ABH antigen recognized on the spermatozoa are inherent (Takeda and Hiraiwa 1985) or merely a coating antigen absorbed from seminal fluid (Scheithauer and Romstöck 1987; Sato et al. 1987). In this study we have examined the localization of the ABH antigens in the male genital tract to try to clarify the situation and showed that the ABO, H, Se, Le and X genes are subjected to a tissue-dependent different expression.

Materials and methods

Specimens of human testis, epididymis, prostate and seminal vesicle were collected at autopsy. The ABO and Lewis blood groups of the tissue donors wee determined from the blood sample by hemagglutination tests. The secretor status of the donors was determined by Lewis blood type and the presence of ABH antigens in the tracheal gland corresponding to that of the donor (Nishi et al. 1989).

Distribution of blood groups of the donors was as followed; A, Le(a+b−): 5, A, Le(a−b+): 29, A, Le(a−b−): 3, B, Le(a+b−): 4, B, Le(a−b+): 4, O, Le(a+b−): 2, O, Le(a−b+): 11, AB, Le(a+b−): 1, AB, Le(a−b+): 2, AB, Le(a−b−): 1.

Monoclonal anti A, B antibody (Seraclone®, Biotest, Germany), monoclonal anti type 1chain $H(H_1)$ antibody (clone no., 17-206, Signet Cambridge, MA, USA), monoclonal anti type 2 chain $H(H_2)$ antibody (clone no. A-503, Dakopatts, Denmark), monoclonal anti Le a, b, x, y antibody (clone no. T174, T218, P12 and F3, respectively, Signet, MA, USA), and anti A_1 antibody being against to type 3 and 4 chain A antigens (Cromatest®, Knickerboker, Barcelona, Spain) were used at dilution of × 50 for immunostaining.

Formalin fixed, paraffin embedded 4 μm sections were deparafinized, immersed in ethanol and pretreated with 0.3% H_2O_2 in methanol for 30 min, followed by 0.1% of bovine serum albumin in 0.5 M Tris-HCl buffer (pH 7.5) containing 0.9% NaCl for 30 min. The tissue sections were incubated with monoclonal antibodies at room temperature for 30 min. Tissue site reacted with monoclonal antibodies were visualized with the Streptavidin Immunostaining kit (Bio Genex, USA). The sections were then counter-stained with hemtoxylin, dehydrated and mounted in balsam (Nishi et al. 1989).

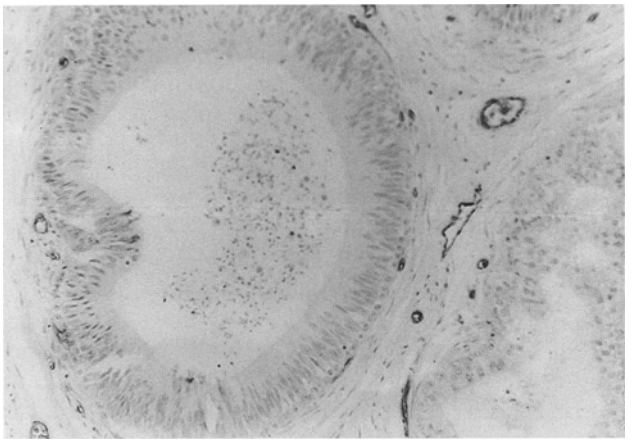


Fig. 1. Ductus epididymidis stained with anti A antibody from blood group A Le(a−b+) individual. Although red blood cells and vascular endothelial cells showed good reactivity with the monoclonal anti A antibody, spermatozoa in the lumen and endothelium of ductus epidydimidis showed no reactivity

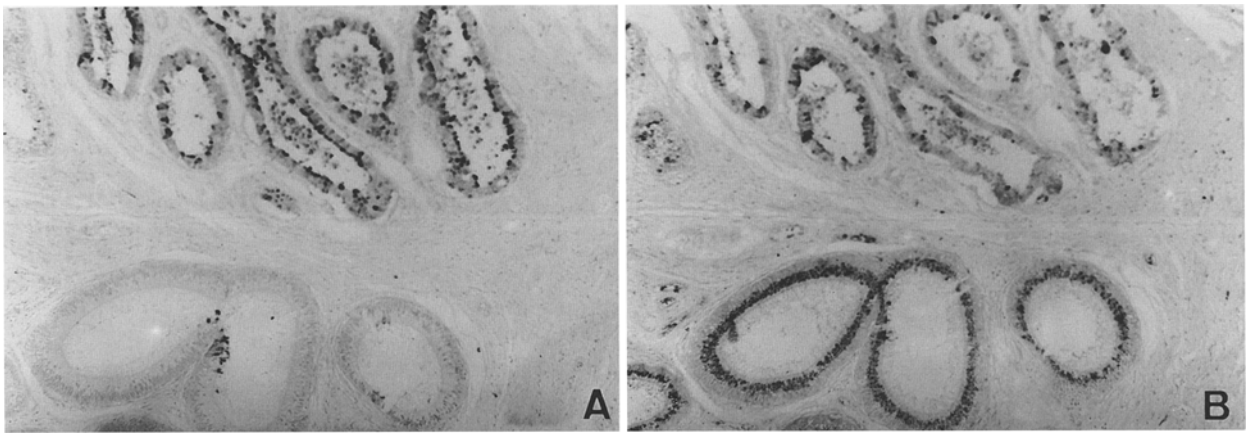
Results

Testis

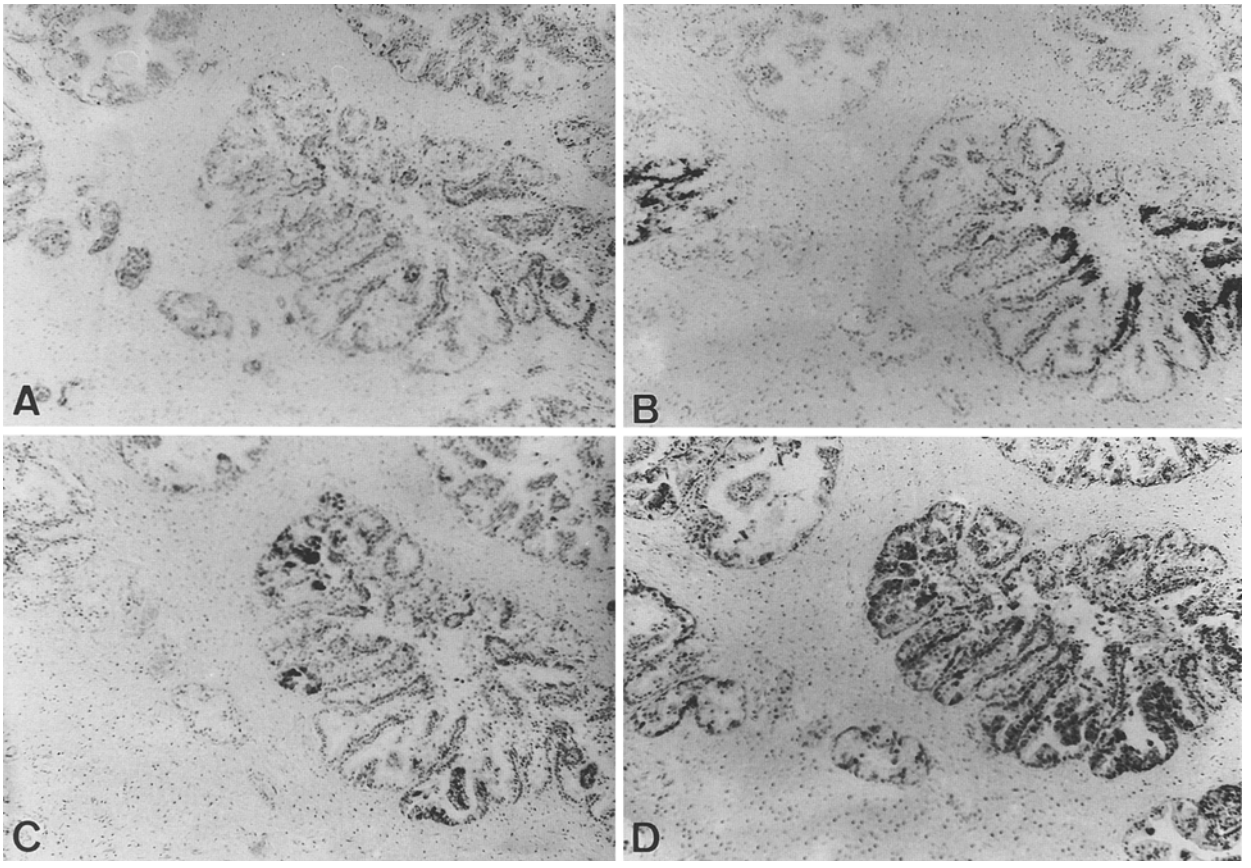
The endothelial cells of blood vessels, erythrocytes and epithelial cells of the rete testis were strongly stained by the corresponding ABH antibodies regardless of secretor status of the donors. Although the precursor cells of the spermatozoa showed weak outlining of the cell envelopes independent of the secretor status, the spermatozoa head observed in the lumen of the testicular canal showed no staining. Leydig cells also showed no reactivity of ABH antigens. Anti A_1 and antibodies against Lewis system did not stain the cell component in the testis.

Ductuli efferentes

The epithelial cells and secreted material in the lumen were strongly stained dependent on the blood group and secretor status of the donors. In Lewis a positive individuals of all ABO groups, anti Le a antibody strongly stained the epithelial cells and secreted material. A small amount of the epithelial cells were also moderately stained by anti Le b antibody. In Lewis b positive individuals of all ABO groups the epithelial cells and secreted material were strongly stained with anti Le b and moderate with anti ABH and Le a. One individual, blood group A(Lea−b−), nonsecretor, showed no reactivity with anti A, Le a and Le b antibodies, whereas the secretory cells of the lingual glands from the same individual produced a small amount of Le a antigen. Although anti Le x antibody showed no reactivity in the ductuli efferentes, anti Le y showed strong reactivity with the epithelial cells and secreted material independent of the blood groups and secretor status of the donors. Anti A_1 antibody showed weak reactivity in the



Figs. 2A, B. Epdidymis stained with anti Le a antibody (**A**) and Le y antibody (**B**) from blood group A Le(a+b−) indiviual. **A** Anti Le a antibody clearly bound to epithelium of ductuli efferentes and feebly to epithelium of ductus epididymidis. **B** On the other hand anti-Le y showed intensive reactivity with the supernuclear portion of the epithelium of ductus epididymidis and the epithelium of ductuli efferentes



Figs. 3A–D. Prostate stained with anti A(**A**), H(**B**), Le a(**C**) and Le y(**D**) antibodies from the same individuals shown in Fig. 1. Erythrocytes and vascular endothelium reacted only with antiA antibody. The epithelium of prostate showed various reactivity with these antibodies showing mosaic distribution of the antigens

supernuclear portion of the epithelial cells from blood group A secretor individuals.

Ductus epididymidis

In ductus epididymidis Le y antibody exclusively showed the strong reactivity in the supernuclear portion, Golgi region, of the epithelial cells irrespective of the ABO blood groups and secretor status of the donors, showing weak or moderate reactivity with anti H_2 in a small amount of the epithelial cells and no reactivity with A, B, Le a and Le b antibodies.

Seminal vesicle

The epithelial cells and secreted material in the lumen from secretor individuals were strongly stained by A and/or B antibody. Anti H_2 antibody showed good reactivity with the cells of all ABO secretor individuals. Anti A_1 antibody showed weak reactivity in the supernuclear portion of the epithelial cells from blood group A secretor individuals. Although anti H_1, Le a, b, and x antibodies showed no reactivity in the seminal vesicle, anti Le y showed strong reactivity irrespective of the ABO blood group and secretor status of the donors.

Prostate

A, B and H antigens were expressed in the epithelial cells and secreted material in the lumen irrespective of the secretor status of the donors and H_2 antigen was expressed in the individuals of all ABO groups. Anti A_1 antibody showed weak reactivity in the supernuclear portion of a small amount of the epithelial cells from blood group A secretor individuals. In Lewis negative individuals anti Le a and b showed no reactivity. In Lewis a positive individuals Le a antibody strongly stained the epithelium whereas Le b antibody staining was weak or negative. In Lewis b positive individuals epithelium strongly reacted with anti Le b, whereas the staining with anti Le a varied from weak to strong positive. Anti Le y strongly stained the epithelium from all individuals examined in this study. Anti Le x showed a feeble staining only in some specimens. However, the distriubtion of the ABH and Lewis antigens in the prostate was very irregular. Although the epithelial cells were strongly stained, the proportion of the positive cells was diverse. In some specimens, only a focal staining was observed, while in others almost all epithelial cells were clearly stained. Furthermore, isolated production of two or three combined A, B and H antigens in several specimens and a typical mosaic distribution of the antigens in a few specimens were also observed. All speci-

Table 1. Localization of ABH related antigens in human male reproductive organs

	Surface of germ cells	Rete testis	Ductuli efferentes	Ductus epididymis	Seminal vesicle	Prostate	Sperm
Antigen							
A, B, H	+	+	+	−	+	+	−
H_1*	−	−	+ (weak)	−	−	+ (weak)	−
H_2*	−	−	+	+ (weak)	+	+	−
Le a, b	−	−	+	−	−	+	−
Le x	−	−	−	−	−	+ (weak)	−
Le y	−	−	+	+	+	+	−
Genes**							
H, Se	H	H	H+Se	H	H+Se	H+Se	
Le, X			Le, X	X	X	Le, X	

* H_1 and H_2 antigens detected in the cells from blood group A, B and AB individuals
** Presumptive genes related to the expression of blood group antigens

mens examined in this study demonstrated relatively small amounts of Lewis a, b and x antigens compared with the ABH and Lewis y antigens.

Discussion

The results obtained in this study suggested that in the human male genital tract ABH related antigens were expressed under the complex and combined gene control as shown in Table 1.

Many investigations have been carried out to attempt to establish a detection method for ABH antigens in seminal stains, especially from spermatozoa (Edwards et al. 1964; Inoue and Okada 1983; Takeda and Hiraiwa 1985; Boettcher 1968; Oyama et al. 1987). It is basically accepted that ABH antigens can be detected on the spermatozoa extracted from seminal stains. However, it has long been a controversial subject whether the ABH antigens on the spermatozoa are inherent to the membrane or coating antigens. Inoue and Okada (1983) reported that ABH antigens on spermatozoa could still be detected after washing 20 times in phosphate buffer. Takeda and Hiraiwa (1985) also reported that the adherence of A or B antigens from saliva or seminal fluid to O type spermatozoa was not observed after incubation in appropriate saliva or seminal fluid and the ABH antigens recognized on spermatozoa are inherent antigens of the sperm. On the other hand, Sato et al. (1987) reported that the amount of ABH antigens on the spermatozoa was reduced when spermatozoa were incubated with tetra-cycline in tissue culture medium and this phenomena results from the capacitation of sperm by tetracycline. They also reported that inactivation spermatozoa showed reactivity with ABH antibody before and after incubation. They claimed from these finding that ABH antigens on spermatozoa are coating antigens in the same way as seminoprotein which is a prostate specific antigen and is adsorbed on to sperm (Akiyama et al. 1984), because many coating antigens which have been detected on spermatozoa are removed by the capacitation of the sperm. In a previous paper (Brinkmann et al. 1986) we reported that although seminal samples showed correlation with the blood group of the donor, very weak or no staining of the spermatozoa membrane was observed and this appeared to be concentration in the extracellular constituents. From the finding of this study, it is not likely that ABH antigens exist in the testicular tissue. Spermatozoa in the testicular canal and ductus epididydimis were not stained by blood group antibodies used in this study. This finding is consistent with that of Szulman (1962) and Lötterle and Heine (1986). The seminal vesicle could express ABH antigens which probably consist of type 2, 3 and 4 chains, under the control by Se, H, A and/or B genes. The prostate gland expressed the ABH related antigens independent of the Se gene. These findings together with our previous report (Brinkmann et al. 1986) indicate that the ABH antigens on the spermatozoa are coating antigens such as α-2-seminoglycoprotein and similar to the Lewis antigens on erythrocytes (Watkins 1974). Tsuda et al. (1988) reported that α-2-seminoglycoprotein isolated from seminal fluid was a seminal vesicle specific antigen, showed moderate reactivity with ABH antibodies and was absorbed by spermatozoa. Scheithauer und Romstöck (1987) reported that spermatozoa were irregularly stained in the ampulla of the vas deferens in which the ABH antigens were secreted independently of secretor status, whereas in the testis and epididymis no reaction could be found. Their findings support the hypothese put forward here and is not inconsistent with previously reported findings (Brinkmann et al. 1986). The spermatozoa in nonsecretor individuals would appear to obtain the ABH antigens from the ampulla ductus deferentis (Scheithauer and Romstöck, 1987) and prostate, and those in secretor individuals from the ampulla ductus deferentis, the prostate and the seminal vesicle. Abrescia et al. (1985) and Dravaland and Joshi (1981) reported the existence of sperm-coating antigens secreted from the epididymis and seminal vesicle. The diverse stainability of the spermatozoa observed in a previous study (Brinkmann et al. 1986) could be due to the relative amount of these adhesive antigens.

Concerning the expression of ABH and related antigens Oriol et al. (1986) proposed a new genetic model which postulated that most tissues of endodermal origin

express ABH and Lewis antigens under control of Se and Le genes, whereas tissues of ecto- or meso-dermal origin express ABH antigens under control of H gene, although there are exceptions to their genetic model. The immunohistochemical studies of Lewis antigens in tissues have been handicapped by both the quality and the range of reagents available. Brunner's glands of the duodenum and deep glands of the stomach, where type 2 chain blood group-related antigens were secreted (Mollicone et al. 1985), were often stained with monoclonal anti Le a and Le b antibodies prepared for the blood grouping of erythrocytes (unpublished observation). Such an unexpected occurence of Lewis antigens may be due to crossreactions of the monoclonal Lewis antibodies with related antigens, such as Le x and Le y antigens. The monoclonal anti Le a, Le b, Le x and Le y antibodies used in this study are highly specific for their corresponding antigens (Cordon-Gardo et al. 1986). In a previous study we found that these reagents gave stable results (Ito et al. 1990). In this study a small amount of the epithelial cells of the ductuli efferentes from Lewis a positive individuals were moderately stained by anti Le b antibody. Many investigators reported that the expression of Le a and Le b antigen is not correlated with the erythrocytes Lewis type of the donors in bladder urothelium (Ørntoft et al. 1991), pancreatic acinar cells (Ito et al. 1990) and sweat duct (Mollicone et al. 1988). Le b antigens are usually expressed in the tissues of glands from Le(a+b−) and occasionally in the tissues from Le (a−b−) donors (unpublished observation).

The results obtained in this study show that expression of blood group ABH related antigens in human male genital tract is controlled by a complex combination of gene systems. The presumptive gene control system is shown in Table 1. In ductuli efferentes of the epididymis, ABH and Lewis a and b antigens were expressed dependent on the secretor status and Lewis phenotype of the donors, however Lewis y antigen was expressed irrespective of the blood group and secretor status of the donors. Furthermore, in the ductus epididymidis, Lewis y antigen was exclusively expressed on the supernuclear portion independent of blood groups and secretor status of the donors. These results indicate that two combined gene systems, that is, one consisting of Se, Le, H and other of H and X genes, relate to the expression of the blood antigens in the ductuli efferentes whereas X and/or H genes code the expression of the Lewis y antigen in ductus epididymidis. In seminal vesicles X and H genes code the expression of Lewis y antigen, and Se and H genes code the type 2, 3, and 4 chain antigens since no Lewis a and b antigens, and type 1 H antigen were observed. However, it is difficult to ascertain whether sialylated Lewis antigens are produced in the epithelial cells of seminal vesicles in this study because the reactivity with anti Le a and Le x were observed without sialidase digestion. In prostate all of the genes probably relate with the expression of the blood group ABH related antigens since ABH and Le y antigens were expressed independently of the secretor status of the donors and Lewis a and b antigens were expressed dependent on the Lewis phenotype of the erythrocytes. Type 3 and/or 4 A chain antigen recognized by anti A_1 are controlled by Se gene and minor component in the human genital tract. In the testicular canal pre-spermatid cells showed reactivity with ABH antibodies in their cell surface and no reactivity was observed on the spermatozoa. This findings suggests that the characteristics of glycoconjugate of the cell surface change during the spermatogenic cell differentiation and maturation, since carbohydrate composition had been found to change during cell differentiation (Hakomori 1981), cell growth (Bremer et al. 1986) and cellular interaction (Blackburn and Schnaar 1983).

In conclusion, the present study indicates that the expression of ABH related antigens in human male genital tract is coded by a combination of several kinds of genes and the carbohydrate character of the germ cell surface is probably changed during cell differentiation.

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