Mucosubstance and Lectin Histochemistry of Cervical Glands in Human Uteri

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INTRODUCTION

Secretory cells of cervical glands in human uterine cervix are known to secrete various mucous substances including those with blood-group specificity(1). Recent studies have shown that lectins conjugated with horseradish peroxidase or colloidal gold are reliable histochemical reagents for demonstrating blood group antigens and their precursor substances in certain human tissues(2,3,4,5,6,7,8). Although labeled lectins and other histochemical methods for mucous substance have been employed for characterizing mucosubstance in cervical glands(9), these previous studies did not pay attention to the dependence of histochemical results on the blood-group of the tissue donors. The purpose of the present study is to examine properties of the cervical mucins, using various histochemical methods including lectin histochemistry at the light and electron microscopic level.

MATERIALS AND METHODS

Human cervical glands were obtained from 36 biopsy and 6 autopsy cases. Tissues were fixed in 10% formalin and embedded in paraffin, and cut at 4μm. For electron microscopic studies, tissues were fixed in one-half Karnovsky's fixative for 2h at 4°C, dehydrated in a graded series of ethanols, and embedded in L.R.White resin(6,7,8). Blood-group typing of the tissue donors was performed by applying routine hemagglutination test. The secretor status of the tissue donor was determined from the Lewis blood type.

1. Staining with Histochemical Methods for Mucosubstances
(1)periodic acid-Schiff(PAS)(demonstration of all poly-and mucosaccharide-containing hexoses and deoxy hexoses with vic-glycol group);(2)alcian blue pH2.5(AB pH2.5)(demonstration of acidic mucosaccharides, mainly sialomucin);(3)alcian blue pH1.0(AB pH1.0)(demonstration of sulphated mucopolysaccharide); (4)AB pH2.5-PAS double staining(AB-PAS)(demonstration of both neutral and acidic mucopolysaccharide in the same sections); (5)high-iron diamine-AB pH2.5 double staining (HID-AB)(demonstration of both sulphated and other acidic mucopolysaccharide in the same sections);(6)aldehyde fuchsin(AF)(demonstration of sulphated mucopolysaccharides);(7)AB-PAS staining after diges-

Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr

tion with neuraminidase(demonstration of sialomucin)
2.Lectin-HRP Staining
Following lectins conjugated with horseradish peroxidase(HRP) were used: Dolichos biflorus agglutinin(DBA), Griffonia simplicifolia agglutinin I-B_4(GSI-B_4), Griffonia simplicifolia agglutinin-II(GS-II), Peanut agglutinin(PNA), Ricinus communis agglutinin-I(RCA-I), soybean agglutinin(SBA) and Ulex europaeus agglutinin-(UEA-I). Blood group ABO and saccharide specificities of these lectins are presented in Table 1. Details of the staining methods have been reported previously(3,4).

Table 1. Lectins used in this study

Lectin	Specificity Human ABO blood group	Sugar	Inhibitory sugar
DBA	A	α-D-GalNAc	D-GalNAc
GSI-B_4	B	α-D-Gal	D-Gal
UEA-I	H(O)	α-L-Fuc	L-Fuc
SBA	None	α/β-D-GalNAc>α/β-D-Gal	D-GalNAc
PNA	None	β-D-Gal(1→3)-D-GalNAc	D-Gal
RCA-I	None	β-D-Gal	D-Gal
GS-II	None	α/β-D-GlcNAc	D-GlcNAc

abbreviations used are GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; Gal, Galactose; Fuc, Fucose.

3. Lectin-gold Complex Staining
Methods for preparing colloidal gold with an average particle diameter of 15nm and lectin(DBA,GSI-B_4,UEA-I)-gold complexes were reported previously(6,7,8). Cytochemical staining procedures were performed according to those reported by Nakajima et al(6,7,8).

RESULTS AND DISCUSSION

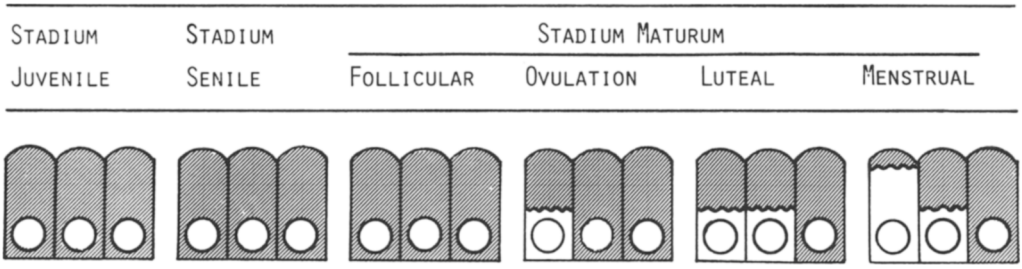
1. Staining with Histochemical Methods for Mucosubstances
The results are summarized in Table 2. Most of secretory cells in the cervical glands were strongly stained by PAS, moderately by AB pH2.5 and weakly by AB pH1.0. They were stained uniformly dark purple by AB-PAS, but in some cases, the basal region of some secretory cells were stained reddish purple by AB-PAS because of weak staining with AB pH2.5 in these regions. HID

Table 2. Stainability of cervical glands in human uteri with histochemical methods for mucosubstances

PAS	AB(2.5)	AB(1.0)	AB-PAS	HID-AB	AF	Neuraminidase AB-PAS
+3	+1~+2	+1~+2	+2~+3	+1~+3	+1~+2	+2~+3

+1, weak staining; +2, moderate staining; +3, strong staining.

Fig 1. Schematic drawing to show the staining patterns of cervical glands with aging and during menstrual cycle



also stained most of secretory cells, however, some cells often did not react with HID and HID-AB sequence resulted in blue tincture of these cells. Similar results were obtained with AF staining. Digestion with neuraminidase did not affect the staining intensity with AB pH2.5. These results demonstrate that cells in the cervical glands secrete mainly neutral and sulphated mucopolysaccharide. Sialic acid susceptible to neuraminidase digestion can not be detected in the present study.
Next, we examined the changes in staining patterns of the cervical glands with aging and during menstrual cycle(Fig.1). The secretory cells in the stadium juvenile and stadium senile were usually stained uniformly by the methods employed. During menstrual cycle the staining patterns changed, depending on the phase of menstrual cycle, namely secretory cells in the follicular phase and the ovulation phase were usually stained uniformly, while, in the luteal phase, many cells exhibited the reactivity with these stains only at the upper half of the cells, and in the menstrual phase, many cells exhibited the reactivity with these stains only in their apical pole.

2.Lectin-HRP Staining

The results are summarized in Table 3. The staining patterns of blood-group specific lectins showed strict dependence on the blood group of the tissue donors. DBA reacted with the cervical glands of blood group A and AB secretors but did not react with those of blood group B and O, and nonsecretors of any blood group. GSI-B_4 also reacted specifically with the glands of blood group B and AB secretors. UEA-I reacted with the cervical glands of any blood group of secretors and weakly reacted with those of blood group B,O,AB nonsecretors but not with those of blood group A nonsecretors. The distribution of cells reactive with these lectins was not homogeneous in blood group A,B,AB donors. For example, in some glands of blood group A donors, only a limited number of cells were stained with DBA, while almost all the cells were stained with UEA-I. Cells, which reacted with DBA but not with UEA-I, were also observed. In blood group B and AB donors, similar mosaic distribution of lectin-reactive cells was observed. Furthermore, in some blood group AB individuals, we often encountered the reverse distribution of DBA and GSI-B_4 positive cells, i.e. some clusters of cells reacted with DBA but not with GSI-B_4 and others which were not stained with DBA, reacted with GSI-B_4.
Blood group nonspecific lectins such as PNA, SBA and RCA-I

Table 3. Lectin-HRP staining of cervical glands in human uteri

Blood group		Case	Lectin used in this study						
			DBA	GSI-B_4	UEA-I	SBA	PNA	RCA-I	GS-II
A	S	17	+1~+2	-~+2	+1~+2	+1~+2	+1~+2	+1	-~+1
	NS	2	-	-	-	-~+2	-~+2	+1~+2	-
B	S	4	-	+1~+2	+2	+1	+1~+2	+1	-
	NS	1	-	-	-~+1	-	+1	+2	-
O	S	11	-	-	+1~+2	-~+2	+1~+2	+1~+2	-~+1
	NS	1	-	-	-~+1	+1	+2	+1	-
AB	S	4	-~+1	+1~+2	-~+2	+1	+2	+1~+2	-~+1
	NS	2	-	-	-~+2	-~+1	+1	+1	-

Numbers indicate the staining intensity. -, no staining; +1, weak; +2, moderate; +3, strong. S, secretor; NS, nonsecretor

reacted with secretory cells regardless of the blood group of the tissue donors. PNA reacted moderately with the cervical glands, while SBA tended to react more strongly with secretory cells of blood group A secretors than those of other blood groups. GS-II did not react with secretory cells of cervical glands.
Generally, these blood group spicific as well as nonspecific lectins reacted uniformly with the secretory cells of the cervical glands of any blood group. However, in luteal phase they tended to exhibit stronger reactivity in the apical pole of the secretory cells. These results suggest that the patterns of lectin staining are likewise dependent on the phase of menstrual cycle as found in conventional mucin staining.
3.Electron Microscopic Observation of Lectin-binding Sites
DBA, GSI-B_4, and UEA-I-gold complexes reacted intensely with the secretory granules(Fig.2). Other cytoplasmic organelles such as nuclei, mitochondria, endoplasmic reticula and the Golgi complexes were not labeled at all with these lectin-gold complexes. Reactivity with these lectin-gold complexes was also strictly dependent on the blood group and secretory status of the tissue donors.
In conclusion, labeled lectins are reliable histochemical reagents for demonstrating the precise distribution of blood group antigens and their related substances at both the light and electron microscopic level in human cervical glands.

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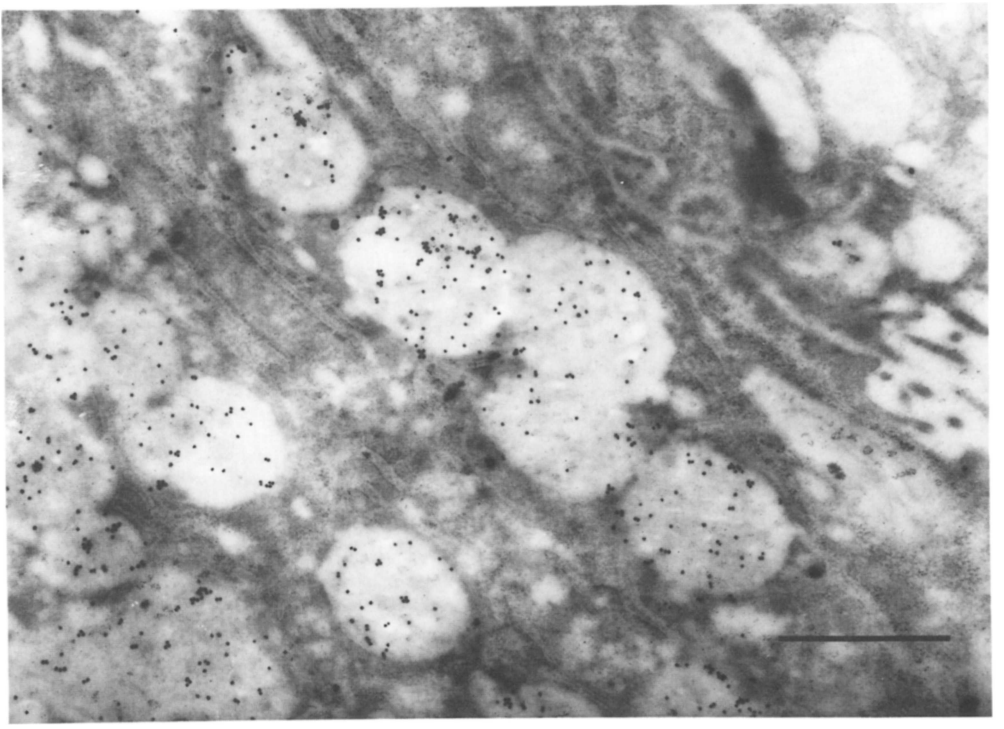

Fig. 2. Secretory cells from a blood-group A secretor incubated with DBA-gold complexes. Secretory granules are intensely labeled. Original magnification x16000, Bar= 1μm.