Monoclonal Antibodies to Blood Group Substances in Vaginal Secretions

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INTRODUCTION

ABO blood grouping of an individual body fluid in a mixture of body fluids is an extremely important problem in medicolegal practices. We are currently carrying out a series of studies to produce monoclonal antibodies (mAbs) to ABO blood group substances (ABO-BGS) in various body fluids, since sandwich ELISA using mAbs to ABO-BGSs in body fluids seems to be the most suitable method for resolving the problem. Consequently, we have already produced mAbs to ABO-BGSs in saliva and semen and we have already applied these mAbs to ABO blood grouping of saliva or semen in a mixture of body fluids by sandwich ELISA (Kimura 1991a,b,c). In the present study, we attempted to produce mAbs to ABO-BGS in vaginal secretions.

MATERIALS AND METHODS

Pooled vaginal swabs (A Se) were extracted with 20mM phosphate buffer, pH 7.4 (PB). The extract was centrifuged and then passed through a membrane filter (0.22μ). The filtrate was applied to a sepharose 4B column (16x200mm) equilibrated with PB and eluated with the same buffer. The fractions carrying A blood group activity were rechromatographed on a sepharose 4B column in the presence of 0.1% SDS. SDS-polyacrylamide gel electrophoresis (SDS -PAGE) and immunoblotting were performed in the conventional manner. Anti-vaginal ABO-BGS mAbs were produced as described previously except that purified vaginal ABO-BGS was used as the immunogen (Kimura 1991a). The procedure for the immunostaining of tissue specimens has been described previously (Ohshima 1991). Sandwich ELISA for ABO blood grouping was performed as described previously (Kimura 1991b).

RESULTS AND DISCUSSION

ABO-BGSs in vaginal secretions (A Se) were analyzed by sepharose 4B chromatography to obtain an immunogen for the production of mAbs to vaginal ABO-BGS. The elution pattern of A blood group activity showed one peak; the Le^b blood group was also found in

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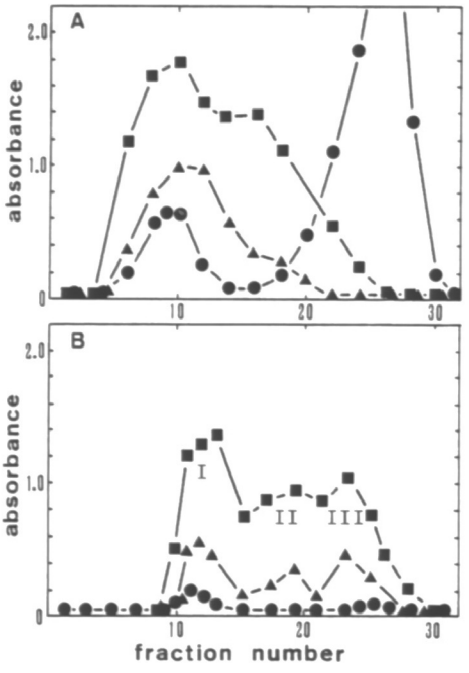

Fig.1. Chromatography of vaginal secretions on sepharose 4B. Fractionation of vaginal secretions was carried out on a sepharose 4B column (A) and then fractions carrying ABO blood group activity were rechromatographed on a sepharose 4B column in the presence of 0.1% SDS (B). Fractions were collected and aliquots were analyzed for protein (●), A blood group activity (■), and Le^b blood group activity (▲).
I, fraction I; II, fraction II; III, fraction III

this peak (Fig. 1A). A and Le^b blood group activities were found in three fractions (fraction I, II, III) on sepharose 4B rechromatography in the presence of SDS (Fig.1B), suggesting that vaginal ABO-BGS consist of at least three components which also carry Lewis blood group activity and which form a complex in vaginal secretions.

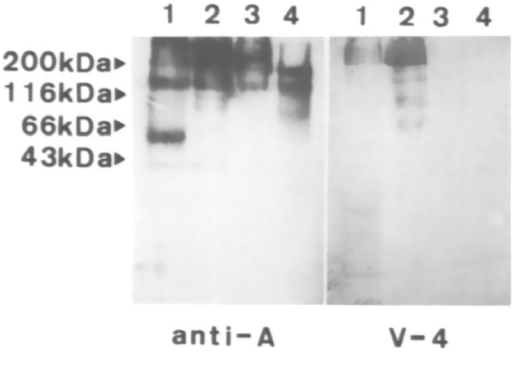

Fig.2. Immunoblotting of fractions containing A blood group activity with anti-A and V-4. whole vaginal secretions and fractions in sepharose 4B rechromatography (Fig. 1B) were separated by SDS-PAGE (5-15% acrylamide gel) and transferred onto a nitrocellulose membrane. The blots were stained with anti-A or V-4. 1, whole vaginal secretions; 2, faction I; 3, fraction II; 4, fraction III

Blood group active fractions obtained by the above chromatographic procedures were analyzed by immunoblotting with anti-A mAb (Fig. 2, left panel). Each fraction consisted of blood group active glycoproteins of over 200 kDa, and about 200kDa and 170 kDa,

respectively. Anti-vaginal ABO-BGS mAbs were produced by using the highest molecular weight component (fraction I) of vaginal ABO-BGS as an immunogen. One (V-4) of the mAbs obtained bound to ABO-BGS in fraction I and to an identical position to that in fraction I in whole vaginal secretions on the blot (Fig. 2, right panel), indicating that V-4 defined the highest molecular weight component of vaginal ABO-BGS. V-4 was specific for vaginal ABO-BGS but not for ABO or Lewis blood groups. Some cells in the cervical glands and almost all the surface columnar cells in formalin fixed tissues of the uterine cervix were stained specifically by V-4, irrespective of ABO blood groups. In the preliminary results only, when V-4 was applied to sandwich ELISA to determine the ABO blood groups in mixtures of body fluids, the ABO blood group of only vaginal secretions was determined (Table 1), although some vaginal secretions derived from blood group O donors showed weak blood group A activity. Further investigations must be carried out to establish the reliability of the present method.

Table 1. ABO blood grouping of body fluids by sandwich ELISA using V-4

specimens	absorbance at 492nm	
	anti-A	anti-B
A Se vaginal secretion (A-Vag)	1.13	0.00
A Se saliva (A-sal)	0.04	0.03
B Se semen (B-sem)	0.00	0.06
A-vag + B-sem	1.20	0.00
A-vag + B-sal	1.16	0.00

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