Production of Monoclonal Antibody to ABH-Carrying α2-Seminoglycoprotein for ABO-Grouping of Semen by Enzyme-Linked Immunosorbent Assay (ELISA)

Y. Tamaki and T. Kishida

Department of Forensic Medicine, Medical College of Oita, Oita 879-56, Japan

INTRODUCTION

Recently, Tsuda et al. (1988) isolated a seminal glycoprotein, A2SGP, with a molecular weight of about 472 000 and identified it as the carrier of ABH epitopes; therefore, it is possible for antibodies to the protein backbone of A2SGP to capture A2SGP in a mixture of semen and vaginal secretions for ABO-grouping of the former without interference of the latter. For that purpose, we produced monoclonal antibodies to the protein backbone of A2SGP and used them in a capture-ELISA.

MATERIALS AND METHODS

A2SGP was isolated from seminal fluids by a sequence of chromatographic procedures as described elsewhere (Tsuda et al. 1988). BALB/c mice were immunized by a subcutaneous injection into hind feet of 20 ug A2SGP in Freund's complete adjuvant. Ten days later, popliteal lymph node cells were fused with P3U1 myeloma cells. Hybridomas were cultured, screened for antibody production by an indirect ELISA in A2SGP-coated plates, cloned, and grown in mice for collection of ascitic fluids, by standard tecniques. The ascitic fluid MAbs were precipitated with ammonium sulfate at 50% saturation and stored at 5 C until use. For ABO-grouping of semen by capture-ELISA, microtiter plates were coated with dilutions of anti-A2SGP MAb in coating buffer. The MAb coats were allowed to react with dilutions of semen. Afer washes, the captured A2SGP was allowed to react with a 1 : 20 dilution of monoclonal anti-A or anti-B or diluent alone. After washes, a 1 : 1000 dilution of horseradish (HRP)-labeled anti-mouse IgM or HRP-anti-H lectin was placed in each well. After incubation and washes, a HRP substrate was added to each well, the enzymic reaction was stopped with 12.5% sulfuric acid, and the plates were read on a microplate reader at 492 nm. All ELISAs were carried out under the following conditions: coating buffer, 0.05 M carbonate, pH 9.5; concentration of coating A2SGP, 10 ug/ml; coating time, 2 h at room temperature; diluent, 0.5% bovine serum albumin in 0.02 M tris-buffered saline conataining 0.1% Tween 20, pH 7.5; washing fluid, running tap water; reaction volume, 100 ul/well; incubation time, 30 min each at room temperature; HRP substrate; 0.005 M *o* -phenylenedamine and 0.001% hydrogen hperoxide in 0.05 M phosphate buffer, pH 5.0.

RESULTS AND DISCUSSION

Four IgG1 MAb-producing hybridoma clones were established. When plates were coated (in a direct immobilization type of ELISA) with semen at 1 : 200

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dilution, the semen coats could be detected with anti-A2SGP at dilutions of 1 : 128 000 to 1 : 512 000. When plates were coated with dilutions of semen, the lower limit of detction with a 1 : 1000 dilution of anti-A2SGP was 1 : 10 240. When tested in a direct immobilization-ELISA, anti-A2SGP did not react with saliva or vaginal secretion coats, although the coats could be detected well with anti-A, anti-B, or anti-H irrespective of secretor status. The results indicate that the anti-A2SGP MAb does not react with ABO epitopes or the proteins of saliva or vaginal secretions; thus, we used the MAb in a capture ELISA to capture exclusively the ABO epitope-carrying protein of semen, A2SGP. Figure 1 shows the results of a capture-ELISA where a semen sample from a group B secretor was captured by anti-A2SGP for subsequent ABO grouping. Even a 1 : 12 800 dilution of semen could be detected and correctly ABO-grouped.

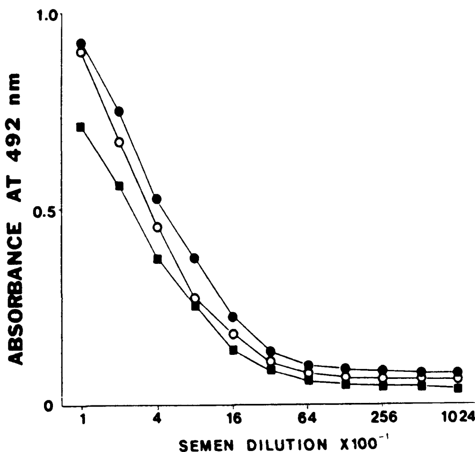

Fig. 1. Capture-ELISA for ABO-grouping of semen. Plates were coated with 1: 200(●), 1:1600(○), or 1:12 800 (■) dilution of anti-A2SGP, and incubated with serial double dilutions of group B secretor's semen. Captured A2SGP was detected with anti-B and HRP-anti-mouse IgM.

One of the major seminal proteins of forensic interest is γ-Sm (Koyanagi et al. 1972) or p30 (Sensabaugh 1978) and has long been used as a marker for semen identification. The present study demonstrates that A2SGP is another sensitive marker when detected by an indirect ELISA method.

Since the individualization of seminal fluids or stains in the investigation of rape cases is the final goal of semen examination in the forensic science laboratory, a number of forensic scientists attempted to ABO-group seminal fluids mixed with vaginal secretions after separation of seminal from vaginal components. Despite their claims of success, they failed to present convincing evidence of complete separation in the absence of information on the ABH substance of semen. Increasingly wide use of ELISA methods in recent years has stimulated a new approach to the ABO-grouping of semen. Using potato lectins or absorbed anti-semen serum in capture (or sandwich) ELISAs, Mukoyama et al. (1987) and Sagisaka et al. (1988) could ABO-group semen mixed with vaginal secretions in all ABO combinations. Tsuda et al. (1988) first isolated A2SGP and identified it as the ABH substance of semen. Immunizing rabbits with A2SGP, Iki et al. (1988) raised anti-A2SGP serum, but they had to absorb it with human red cells and serum before use. Although monospecific, absorbed anti-A2SGP serum may pose problems in ELISAs, such as crossreaction and nonspecifc

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adsorption. To avoid the potential hazard of crossreaction, we produced anti-A2SGP MAbs of well-defined specificity. These MAbs worked well in the capture ELISA described here; however, for practical applications they need extensive testing with mixed body fluids and stains.

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