PURIFICATION OF MOUSE MONOCLONAL ANTI-HCG IgG₁ FROM ASCITES FLUID BY HYDROXYLAPATITE CHROMATOGRAPHY.

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SUMMARY

Mouse monoclonal IgG₁ antibody to human chorionic gonadotropin from ascites fluid was purified by one-step hydroxylapatite chromatography. The purified monoclonal antibody is over 90% yield and essentially free of contaminating mouse IgG found in ascites fluid.

INTRODUCTION

Mouse ascites fluid generated by injection of hybridoma cells contains numerous other proteins, in addition to the desired monoclonal antibody produced by the hybridoma cells. The usage of monoclonal antibodies in vitro and in vivo diagnostic and therapeutic agents now require highly purified reagents free of contamination with other proteins or biologic molecules.

Although protein A-Sepharose chromatography method has been used most commonly for the separation of IgG subclasses from various sources, such as mouse serum, ascites fluid and human serum (Ey et al., 1978; Seppala et al., 1981; Vilemez et al., 1984; Stephenson et al., 1984; Patrick and Virella, 1978), other methods of purification must be used for the purification of mouse monoclonal IgG₁ antibodies that apparently do not interact with protein A (Villemez et al., 1984). Immunoaffinity chromatography methods are highly specific, but harsh conditions are usually necessary to break antigen-antibody complexes, which often result in inactivation of the monoclonal antibody. Hydroxylapatite chromatography methods to purify
monoclonal antibody on the basis of light-chain composition variations were reported recently (Stephenson et al., 1984; Patrick and Virella, 1978). But the above methods are not apparently confined to any given subtype of IgG₁ monoclonal antibody.

This report describes the simple and rapid purification of monoclonal IgG₁ subclass to human chorionic gonadotropin (HCG) from mouse ascites by the use of hydroxylapatite chromatography.

MATERIALS AND METHODS

Reagents  HCG was obtained from Sigma (St. Louis, MO 63178 U.S.A.). Rabbit anti-mouse-IgG-horseradish peroxidase conjugate, goat antiserum to mouse IgG₁, IgG₂A, IgG₂b and IgG₃ were obtained from ICN ImmunoBiologicals (Lisle, IL 60532 U.S.A.). Goat antiserum to mouse IgG was obtained from Bio-Yeda (Weizman, Israel). Hydroxylapatite was purchased from Bio-Rad (Richmond, CA 94804 U.S.A.).

Hybridoma  The hybridoma cell line (2F-7) producing whole HCG-specific monoclonal antibody were previously developed in our laboratory (Cha et al., 1987). BALB/c mice were injected intraperitoneally (i.p.) with 0.5 ml pristane 1 week before i.p. inoculation of 2 × 10⁷ hybridoma cells.

Hydroxylapatite chromatography  Ascites fluids were centrifuged at 20,000 x g for 30 min at 4°C prior to chromatographic separation on a hydroxylapatite column. Six ml of ascites fluid was applied on a column of hydroxylapatite (1.8 x 36 cm) hydrated in buffer A (0.01 M sodium phosphate buffer containing 0.02% sodium azide, pH 6.8). The bound proteins were then eluted with a 300 ml linear phosphate gradient (pH 6.8) between 0.01 M and 0.3 M at room temperature. The flow rate was 10 ml/hr and fractions of 100 drops were collected. The eluent absorbance was monitored (SP 8-100, PYE UNICAM) at 280 nm. The fractions were pooled according to the chromatogram peaks.

Antibody assay  The activities of anti-HCG antibody and mouse antibodies in the column fractions were assayed using an enzyme-linked immunosorbent assay (ELISA) (Caraux et al., 1985).

Characterization and IgG subclass identification  For the IgG characterization or subclass identification, the method of immunodiffusion and immunoelectrophoresis were used. For the immunodiffusion, the well of 1% agarose gel plates were filled with fraction sample and reagent antiserum to mouse IgG₁, IgG₂A, IgG₂b and IgG₃. The plates were incubated in a saturated humid chamber at room temperature for 48 hrs before reading and diffused in saline containing 0.02% NaN₃ over 3 days. Immunoelectrophoresis was performed under the constant voltage, 200 volt on 4°C using plate, Gel Bond Film (84 x 94 mm), for 2 hrs with 0.02% sodium azide. After the electrophoresis, the plates were diffused in saline for 3 days and stained in 1% coomassie brilliant blue R-250 prepared in destaining solution (MeOH : CH₃COOH : H₂O = 45 : 10 : 45) at room temperature for 2 hrs and the destaining was carried out with the destaining solution until the background disappeared.