Production and Characterization of Monoclonal Antibody against Infectious Hematopoietic Necrosis Virus

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A hybridoma cell line that continually secretes antibody against infectious hematopoietic necrosis virus was developed and designated M-IHNV-W1. Antibodies in culture fluids were concentrated and purified by Protein A Sepharose CL-4B affinity chromatography. The immunoglobulin was isotyped as IgG 2b with a kappa light chain. The IgG was specific for infectious hematopoietic necrosis virus and had binding activity but low neutralizing activity. Little cross-reactivity occurred with viral hemorrhagic septicemia or infectious pancreatic necrosis viruses.

Introduction

Infectious hematopoietic necrosis, a viral disease of salmonid fishes, has caused severe economic losses since the early 1950's in the northwestern United States (McALLISTER, 1979). Previous attempts to develop antisera to infectious hematopoietic necrosis virus (IHNV) animals have shown the virus to be a particularly weak immunogen. With the development of hybridoma technology, an alternative to antiserum production in animals became available for producing specific immunologic reagents (DIAMOND *et al.*, 1981). We describe here the generation of a hybridoma cell line that secretes antibody specific to IHN virus. Monoclonal antibody was characterized as to immunoglobulin class and subclass, viral specificity, and neutralizing or binding activity.

Materials and Methods

Immunization

The Oregon strain of IHNV was concentrated and purified from chinook salmon embryo cells (CHSE-214) by the method of de KINKELIN (1972). BALB/c mice were injected intraperitoneally (i.p.) with 0.2 ml of a 1:1 mixture of purified virus (100 µg viral protein) and Freund's complete adjuvant.

Subsequently, three i.p. booster injections (25 µg IHNV/0.1 ml) without adjuvant were administered at 10-day intervals. Serum antibody levels were monitored using an avidin-biotin based enzyme linked immunosorbent assay (ELISA). The ELISA system employed was obtained from Vector Laboratories, Burlingame, California.

Myeloma Cells

The mouse myeloma cell line P3-X6430-Ag 8.653 was selected as the fusion partner because it lacks host immunoglobulin expression and has a high fusion ratio (KEARNEY *et al.*, 1979). Cells were maintained in a 1:1 mixture of RPMI-1640 and Dulbecco's Modified Eagle Medium containing 20 mM dextrose, 35 mM NaHCO₃, 15% fetal bovine serum, and 100 µg/ml gentamycin (RDGS). Cells were cultured at 37°C in a 10% CO₂ atmosphere.

Cell Fusion and Cloning

We fused splenic lymphocytes from IHNV immunized mice with mouse myeloma cells 3 days after the last booster injection using the procedure described by GODING (1980). Following the fusion, cells were diluted in RDGS containing 100 µM hypoxanthine, 2 µM aminoprotein, and 30 µM thymidine, dispersed into 96- and 24-well plates, and incubated at 37°C in a 10% CO₂ atmosphere.

(GODING, 1980). Hybrid cell populations began to appear 3–4 weeks postfusion, and the populations were screened for antibody production by ELISA.

The hybrid cells were cloned using a modified limiting dilution procedure. Cell were transferred from a well by vigorous aspiration and placed in well 1A of a new 96-well plate, on which each well contained 0.1 ml of feeder cells (nonimmunized BALB/c mouse spleen cells in RDGS). Serial two-fold dilutions were made transferring 0.1 ml of cells horizontally, and the wells were brought to 0.2 ml by adding feeder cells. Then, serially twofold dilutions were made vertically transferring 0.1 ml of cells, and all wells were brought to 0.2 ml by adding feeder cells. Cultures were incubated at 37°C in a 10% CO₂ atmosphere. Hybrid populations underwent at least three clonings before they were transferred to larger culture vessels.

Production of Ascitic Fluid and Tumors

BALB/c mice were primed by i.p. injection of 0.5 ml of pristane. After 5 days, primed and non-primed mice were injected i.p. or subcutaneously with approximately 2.0×10^6 fused cells in 0.2 ml of RDGS. Ascites accumulated and tumors developed 10–30 days postinjection in both groups of mice.

Concentration and Purification of Antibody

Antibody was concentrated and purified from cell culture medium using Protein A Sepharose CL-4B affinity chromatography as described by GOOD *et al.* (1980).

Antibody Characterization

Immunoglobulin class and subclass were determined by using a commercially available assay system (Boehringer Mannheim, Indianapolis, Indiana). Neutralizing and binding activities were determined by standard neutralization procedures (McDANIEL, 1979) and ELISA, respectively. Specificity of the monoclonal antibody was determined by ELISA testing against infectious pancreatic necrosis virus (IPNV, serotype VR-299), viral hemorrhagic septicemia virus (VHSV-F1, F2, 23.75 serotypes) and Freund's complete adjuvant. Wells were coated with approximately 500 ng of the respective viruses or with undiluted Freund's adjuvant.

Cryopreservation of Hybrid Cells

Hybrid cells were frozen in liquid nitrogen at a concentration of 2.0×10^7 cells/ml. The freezing medium consisted of Dulbecco's minimum essential medium containing 15% fetal bovine serum and 7.5% dimethylsulfoxide as the cryopreservative (GODING, 1980).

Results and Discussion

The original antibody producing cell population was cloned at least six times, resulting in numerous hybridoma cell cultures. More than 99% of the cloned cultures secreted antibody to IHNV as detected by ELISA. Hybrid cell lines were considered

Fig. 1. Elution profile of IgG concentrated and purified from hybridoma cell culture medium by Protein A Sepharose CL-4B affinity chromatography.

Fig. 2. Specificity of monoclonal antibody for IHNV. *Culture fluids from cells recovered after 2 months storage in liquid nitrogen.

to be stable after three to six clonings. Several clones were selected for expansion and have been passed more than 70 times. The cell line was designated M-IHNV-W1, to indicate a monoclonal antibody secreting cell line against whole IHNV.

Attempts to obtain higher levels of antibody by inducing the production of tumors and ascitic fluid in BALB/c mice were unsuccessful. Tumor cells reestablished in culture showed no reduction in growth rate; however, antibody production was not stimulated to a greater degree. Similarly, ascitic fluids harvested to date have not contained higher concentrations of antibody than those produced by the original hybridomas. Therefore, cell culture fluids remain the most reliable source of antibody. About 10–16 mg of purified immunoglobulin can be recovered from 400 to 500 ml of culture fluid (Fig. 1). Optimum amounts of antibody are obtained when cell culture fluids are harvested every 3 days. The immunoglobulin produced by hybrid cells was class IgG, subclass 2b, with a kappa light chain. Results of ELISA showed that the IgG bound specifically to IHNV and did not cross-react with IPNV, VHSV, or Freund's complete adjuvant (Fig. 2). Neutralization assays demonstrated that purified IgG (1 mg/ml) had a titer of 1:50 against 50 TCID₅₀ of IHNV, indicating that IgG binds but does not significantly neutralize the virus.

Hybridoma cultures that were recovered after being frozen in liquid nitrogen showed no decrease in growth rate or antibody production (Fig. 2). Cultures passaged more than 70 times have been recloned with no evidence of reversion. The hybridoma cell line M-IHNV-W1 is now being routinely used for production of IgG against IHNV.

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