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Culturing of Interspecies Hybridomas in Immunologically Tolerant Animals

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A new method of culturing monoclonal antibody-producing rat—mouse hybridomas in mice tolerant to the transplantation antigens of rats is described.

Key Words: *hybridoma; xenotolerance; monoclonal antibodies; mouse α-fetoprotein*

Generally, hybridomas are produced by fusing lymphocytes from immunized mice with mouse myeloma cells [6]. Since most myelomas were induced in BALB/c mice and, consequently, have H-2^d haplotype, hybridomas are propagated in BALB/c or nude mice, a strain derived from BALB/c mice. However, sometimes interspecies hybridomas, for example, rat—mouse, are required for production of monoclonal antibodies (MAB) against mouse antigens. These hybridomas can be maintained in a cell culture, which is associated with low efficiency and high risk of contamination with mycoplasma, or in immunodeficient mice (Scid or nude), which requires special conditions of breeding and maintenance.

Here we propose a new method of growing interspecies hybridomas (rat—mouse) in mice tolerant to rat antigens.

The approach is based on a simple method of inducting tolerance to rat grafts in adult mice [3,4]: one month after thymectomy [7], a suspension of August rat splenocytes is intravenously injected into mice (1.2×10^8 cells/mouse), and cyclophosphamide (200 mg/kg) is injected intraperitoneally after 24 h. Previously, we showed that this treatment provides prolonged tolerance to xenografts (rat heart, urinary bladder epithelium, and tumor cells) in 70% of mice [5].

On the basis of this approach we have developed a technique for growing rat—mice or man—mice hybridomas in immunologically tolerant mice.

MATERIALS AND METHODS

Hybridomas were grown in 35 BALB/c mice (H-2^d) and (DBA×BALB/c) F_1 mice. Tolerance to August rat cells (RT1^c) was induced at the age of 2-3 months. Several schemes of tolerance induction were used. Hybridoma cells (10^6-10^7 per mouse) were injected intraperitoneally simultaneously with rat splenocytes or 3-4 h after cyclophosphamide. Two hybridomas producing MAB to mouse α-fetoprotein (mAFP, clones D_6 and G_4) and one hybridoma producing MAB to the allotypes on rat immunoglobulin heavy chains (clone C3B9) were selected for experiments.

D_6 and G_4 hybridomas were obtained as described elsewhere [6] by fusing splenocytes of immune rats (mAFP) and X63Ag8.653 mouse myeloma cells. Anti-mAFP monoclonal antibodies secreted by D_6 and G_4 hybridomas were isotype 2a rat IgG distinguishing two individual epitopes of mAFP [2,8].

C3B9 hybridoma producing MAB to the allotypes on heavy chains of rat immunoglobulins was

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obtained at the Institute for Industrial Selection of Organisms. The hybridomas were cultured in flasks (Nunclone) in RPMI-1640 or DMEM medium (Sigma) supplemented with 1% L-glutamine and 15% fetal calf serum (Flow Lab.), 5×10^{-5} M 2-mercaptoethanol, and 0.05 M HEPES.

Hybridoma cells were suspended in the culture medium, centrifuged for 5 min at 1200 rpm, the pellet was resuspended in serum-free culture medium, cells were counted, and the suspension was injected into mice in an intraperitoneal dose of 10^6-10^7 cells per animal. The anti-mAFP activity in the supernatant was measured in an immunoenzyme assay.

One week prior to transplantation of hybridomas, some mice were injected with 1 ml sterile Vaseline oil.

Anti-mAFP MAB in hybridoma-conditioned culture medium and ascites fluid or peritoneal lavage from hybridoma-carrying mice were identified by modified solid-phase immunoenzyme assay [1,9]. Culture media were analyzed without diluting and at 5-fold serial dilutions from 1:5 to 1:3215. Mouse sera, lavages, and ascites were tested in 10-fold dilutions to 10^{-7}. Unconditioned culture medium or phosphate-buffered saline were used as negative controls.

The reaction was performed in a volume of 100 µl. Purified mAFP (1 µg/ml) was immobilized on plastic (Nunc-Immuno microplates) and incubated with culture medium, ascitic fluid, or peritoneal lavage after blockade of nonspecific binding sites with 0.5% human serum albumin in phosphate-buffered saline with 0.01% Tween-20. Bound anti-mAFP MAB were revealed with rabbit anti-rat IgG conjugated to horseradish peroxidase. After incubation with the substrate (0.8 mg/ml 5'-aminosalicylic acid, pH 6.0, 0.005% H_2O_2), light absorbance was measured at 450 nm in a micro-ELISA Minireader MR-590 (Dynatech).

RESULTS

The protocol in which the induction of tolerance to xenogeneic cells (thymectomy, intravenous administration of rat splenocytes 1 month after surgery, and intraperitoneal injection of 200 mg/kg cyclophosphamide after 24 h) is followed by injection of 10^6-10^7 hybridoma cells 3-4 h after a single injection of cyclophosphamide yielded better results than the protocol in which hybridoma cells are inoculated 2 weeks or 1 month after cyclophosphamide injection effective. The last injection of hybridoma cells in the first protocol induced tolerance and served as a test-injection, after which no additional inoculation of hybridoma cells was necessary.

In 13 out of 35 tolerant BALB/c and (DBA× BALB/c) F_1 mice injected with 10^7 D_6 cells, hybridoma grew as ascitic or solid tumor. Anti-mAFP antibodies were revealed by ELISA in the serum and ascitic fluid of mice in which hybridoma growth was seen with the unaided eye (Figs. 1, 2). In 6 out of 12 mice without visual hybridoma growth, specific anti-mAFP activity was detected in peritoneal lavage, indicating production of MAB by hybridoma cells in these mice (Fig. 3).

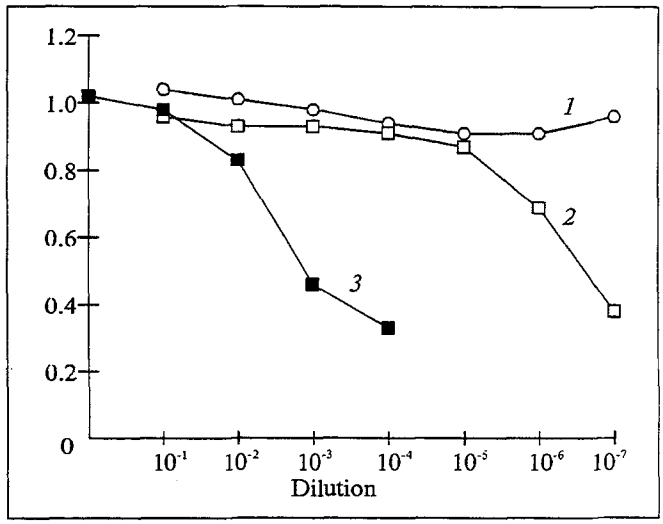

Fig. 1. Specific activity of monoclonal antibodies to mouse α-fetoprotein in serum and ascitic fluid of tolerant mice carrying D_6 hybridoma. *1)* serum; *2)* ascitic fluid; *3)* hybridoma-conditioned culture medium. Here and in Figs. 2 and 3: ordinate: light absorbance at 450 nm in solid-phase immunoenzyme assay.

Administration of Vaseline oil to tolerant mice one week prior to injection of D_6 cells substantially increased the content of MAB in peritoneal lavage (Fig. 3).

Rapid growth of ascitic tumor (a 20-fold increase) after administration of 10^7 G_4 cells led to

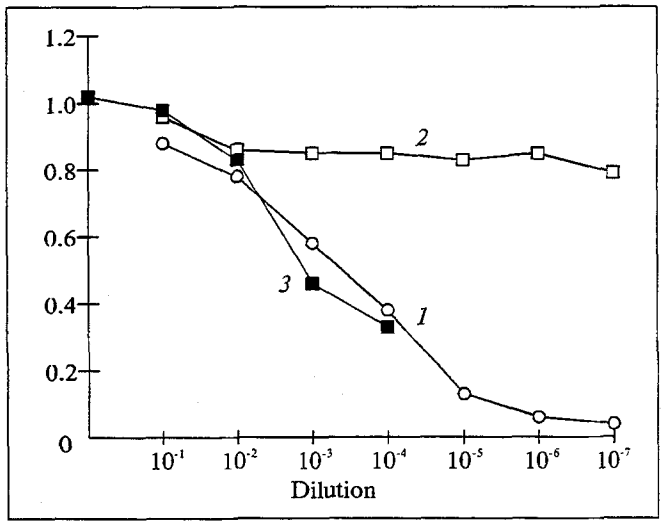

Fig. 2. Specific activity of monoclonal antibodies to mouse α-fetoprotein in serum of tolerant mice with growing D_6 hybridoma. *1)* serum No. 1; *2)* serum No. 2; *3)* hybridoma-conditioned culture medium.

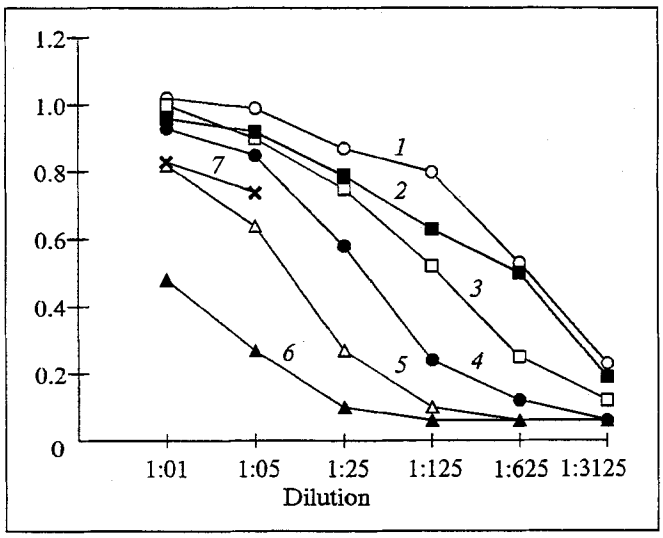

Fig. 3. Specific activity of monoclonal antibodies to mouse α-fetoprotein in peritoneal lavage serum and ascitic fluid of tolerant mice carrying D_6 hybridoma. *1-3*) peritoneal lavages from mice with D_6 hybridoma after injection of Vaseline oil; *4-6*) without Vaseline oil; *7*) hybridoma-conditioned culture medium.

death of all 12 mice. Rapidly growing soft tumor consisting of round cells with large nucleus characteristic of the C3B9 hybridoma cells developed in the abdominal cavity in 8 out of 14 mice injected with C3B9 hybridoma cells producing MAB to the allotypes on heavy chains of rat immunoglobulins. Peritoneal lavage of these mice was not analyzed for anti-mAFP activity.

Thus, a new method of culturing interspecies hybridomas in tolerant mice has been developed. This method is much cheaper than hybridoma propagation in nude mice and provides sufficient amounts of MAB-producing cells.

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