

EMORY UNIVERSITY
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Guidelines: Monoclonal Antibody Production

OVERVIEW:

Monoclonal antibodies (MAb) are a vital tool in medicine and scientific research.. They are produced by fusing a tumor cell with a specific antibody-producing cell (splenic lymphocyte) to produce a cloned cell (hybridoma) which secretes large quantities of a single antibody type with a single binding specificity and affinity (monoclonal). These guidelines must be followed when rodents are used to produce MAb.

In vivo hybridoma technology involves three general phases: 1) immunization of splenocyte donor rodents, 2) preparation and seeding of hybridoma recipient rodents, and 3) collection of ascites fluid.

Splenocyte donor rodents are immunized with an antigen (with or without an adjuvant) via one or two intraperitoneal (IP) or subcutaneous (SC) injections. Often, a final intravenous booster of antigen in saline is given shortly before spleen cell harvest. Harvested splenic lymphocytes are then fused in vitro to myeloma cells and grown in cell microcultures. After growth, the lymphocyte microcultures are screened for monoclonal production and positive cultures are grown to larger quantity. Subsequently, histocompatible hybridoma recipient animals are given one or more intraperitoneal injections of a priming agent (pristine, Freund's complete adjuvant, Freund's incomplete adjuvant) 7-10 days prior to peritoneal seeding with hybridoma cells. After seeding, ascites fluid begins to accumulate within 5-14 days and is collected by insertion of a sterile needle into the peritoneal cavity (paracentesis). The collected antibody is then purified.

GUIDELINES:

- I. Immunization of Splenocyte Donor Mice
 - A. Standard Protocol
 1. Rodents (rats, mice) should be immunized initially with 15-125 mcg of antigen mixed with adjuvant injected subcutaneously at the tail base or given intraperitoneally.
 2. One month after the initial immunization, the animals should be given a booster injection.
 3. Three days prior to euthanasia for spleen harvest, antigen may be administered intravenously in saline (0.1-0.2 ml volume) to stimulate the immune system.
 - B. Considerations
 1. Adjuvants:
 - a. The following adjuvants are acceptable for initial immunization: alum, Ribi Adjuvant System (RAS),

synthetic block copolymers, squalene, incomplete Freund's adjuvant (IFA), complete Freund's adjuvant (CFA). CFA should be administered once per animal at a total volume of 0.1 ml IP or two 0.05 ml injections subcutaneously in each flank.

- b. The following adjuvants are acceptable for boosting: alum, RAS, synthetic block copolymers, squalene, IFA. Complete Freund's adjuvant is not approved for boosting and may not be injected more than once per animal. If IFA is used, it should be given 0.1 ml IP or 0.05 ml SC in each flank.

2. Multiple Boosters:

- a. Boosters may be given beginning 2-4 weeks after initial immunization and at 2 week intervals for up to 6 total injections.

3. Blood Collection for Antibody Titer:

- a. If serum is collected for antibody screening, the mice must be anesthetized (methoxyflurane is the agent of choice) prior to blood collection.

II. Preparation and Seeding of Hybridoma Recipient Mice

A. Standard Protocol-Ascites Production

1. Animals should be primed once with an intraperitoneal injection of 0.2-0.5 ml pristine (2,6,10,14-tetramethylpentadecane).
2. Following priming, the animals are rested for 10-30 days (10-14 days recommended).
3. Following the rest period, hybridoma cells in 0.1-0.5 ml Dulbecco's Modified Eagle's Medium (DMEM) are injected IP.

B. Considerations

1. Priming Agents: Mineral oil, IFA, CFA, and antigen-antibody complexes have been used to induce/enhance ascites, but pristine has proven to be the most consistent and reliable agent.
2. Hybridoma Preparation: In general, the best success in hybridoma production occurs when tumors are grown in vivo and then adapted to tissue culture.
3. Hybridoma Inoculum: The numbers of cells required for ascites production vary somewhat with tumor type. It is recommended that a minimum of 10^6 cells, but, preferably, 10^7 cells be seeded intraperitoneally.
4. Hybridoma Recipients: Histocompatible hybridoma recipients are required. Protocols requiring interspecies monoclonal production or nonhistocompatible animals must be essential for the research and specifically justified.

III. Monitoring and Collection of Ascites Fluid

A. Standard Protocol

1. Animals must be monitored daily by the investigator for abdominal distension following hybridoma seeding.
2. When abdominal distension is evident, the animals should be anesthetized with methoxyflurane and ascites fluid can be collected by paracentesis using a sterile 20 gauge needle.
3. Animals must continue to be monitored and paracentesis may be performed a second time (usually 3-5 days later) as a terminal procedure when abdominal distension recurs.

B. Considerations

1. Using these procedures one should expect to obtain 5-10 ml of high titered ascites fluid per mouse and 30-50 ml per rat.
2. Abdominal distension secondary to ascites accumulation typically becomes evident 5-14 days after hybridoma seeding. Animals must be checked daily (including weekends) during this time.

IV. Euthanasia Criteria

- A. Where possible, all animals must be euthanized within 28 days of hybridoma injection.
- B. Any animals that exhibit signs of discomfort such as weight loss, hunched posture, anorexia, lethargy, or piloerection must be euthanized at that time.

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