Guidelines for Ascites Production in Mice

In vitro methods are to be used for the production of monoclonal antibodies (MAb) unless there are clear scientific reasons why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product.[6] Examples of scientific reasons for justification of the use of the ascites method that Institute/Center (IC) Animal Care and Use Committees (ACUC) may find acceptable are listed in Table 1. References describing alternatives to in vivo methods of monoclonal antibody production are available.[1,3,7-9] When the mouse ascites method for producing MAb is required, every reasonable effort should be made to minimize pain or distress, including frequent observation of the animal, limiting the number of survival abdominal wall punctures, and prompt euthanasia if signs of distress appear.[2] It is the responsibility of the IC ACUCs to determine whether animal use is required for scientific or regulatory reasons.[6]

The specific guidelines for consideration by Principal Investigators when developing animal study proposals and for ACUCs when reviewing proposals involving the mouse ascites method are:

1. Biological material and animal products such as cell lines, tissues, and tumors that are introduced into research animals can harbor animal pathogens (e.g. ectromelia, lymphocytic choriomeningitis, mouse hepatitis and others) which can then infect NIH animal colonies. Principal Investigators are responsible for ensuring that the biologic materials used in their study will not endanger the health of the live animals used in their study, other animals housed in the animal facility, or personnel. Hybridomas must be tested with a PCR-based or species-specific antibody production assay before introduction into an animal host unless otherwise approved by the Animal Program Director (APD), or their designee - generally the IC Rodent Import Officer (RIO) or the Facility Veterinarian. The APD or their designee must review and approve the test results prior to Investigator use of the hybridoma cells on an approved animal study proposal which includes the ascites production being considered. Hybridomas tested years ago with incomplete pathogen-target sets should be re-tested. Hybridomas obtained from other investigators on campus must be approved by the APD or their designee prior to use even if they have been previously used in animals at NIH. This is required because of variations in the health status between different facilities and even within facilities from room to room.

   Reminder: Any rodent-derived product, not just hybridomas, introduced into animals must be approved on the Animal Study Proposal (ASP) and known to be specific pathogen free. Laboratory personnel must always be aware of any laboratory practices that could potentially transmit an infectious agent into an animal facility. For example: antibodies/serum must be approved free of infectious agents if they are to be injected into animals or used *in vitro* to treat cells that will be transferred *in vivo.*

2. The generation of ascites fluid requires the use of a "priming" agent. The most common "priming" agent used is Pristane but Freund's Incomplete Adjuvant has also been shown to be effective at doses of 0.25 or 0.5 ml and could produce superior results. Concern has been expressed about the potential for discomfort and distress that may be associated with "priming" agents, particularly Pristane.[6] Due to this concern, many guidelines suggest a lower 0.1 to 0.2 ml dose of Pristane instead of a higher 0.5 ml dose.[2,3,5,6,9] Therefore, consideration for using a lower dose is strongly encouraged. However, it is also recognized that, as an ILAR report states, "in some strains of mice, 0.2 ml might not be sufficient to produce ascites and that as much as 0.5 ml might be required."[6]

3. The number of hybridoma cells inoculated and the inoculum volume commonly range from 10^5 -10^7 cells in volumes of 0.1 - 0.5 ml, but may vary with the hybridoma line.[5] The standard time interval between priming and inoculation of hybridoma cells is 10-14 days after priming. Generally, very high cell numbers are associated with greater morbidity, and less than 1 x 10^5 cells may elicit fewer ascitic tumors. Cell suspensions must be prepared according to the ARAC Guidelines for Use of Preservative-Free Pharmaceuticals and Parenteral Fluids in Laboratory Animals.

4. Once hybridoma cells are injected, animals should be monitored at least once daily, seven days a week by personnel familiar with clinical signs associated with ascites production.

5. Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort or interfere with normal activity. Manual restraint or anesthesia may be used for abdominal paracentesis (peritoneocentesis or 'tapping'). The tap should be performed by trained personnel using proper aseptic technique. The smallest needle possible that allows for good flow (18-22 gauge) should be used. Fluid should be removed slowly and allowed to drip from the hub of the needle rather than be aspirated with a syringe. [4,6]

6. Animal(s) should be monitored frequently over several hours following the tap to observe possible signs of shock due to fluid withdrawal. Animals should continue to be monitored at least once daily, seven days a week by personnel familiar with clinical signs associated with ascites production, circulatory shock, pain, and distress. Pale eyes, ears and muzzle and breathing difficulties are indicative of circulatory shock. Shock may be prevented or treated by replacement of the volume removed with physiological fluids, typically two to three ml warm saline or lactated ringers administered as one ml by the intraperitoneal route and the remaining volume by the subcutaneous route. Signs of pain or distress may include hunched posture, rough hair coat, reduced food consumption, loss of body condition, emaciation, inactivity, difficulty in ambulation,

respiratory problems, and palpable solid tumor growth. Animals should be euthanized promptly before the final tap or if there is evidence of hemorrhagic paracentesis, pain, or distress.

7. The number of taps should be limited, based on good body condition of the animal. A maximum of three survival taps (the fourth being terminal) are recommended. Additional taps should have ACUC approval.

Table 1
Examples of scientific reasons for the use of the ascites method.[6]

a.	The hybridoma cell line will not adapt well to *in vitro* conditions.
b.	In applications where several different mouse MAb at high concentrations are required for injection into mice, the *in vitro* method can be inefficient
c.	MAb from mouse ascitic fluids might be essential for experiments in which MAb are used *in vivo* in mice
d.	Rat hybridoma cell lines do not generate ascites efficiently in rats, and usually adapt poorly to *in vitro* conditions, but usually generate ascites in immunocompromised mice.
e.	Downstream purification can lead to protein denaturation and decreased antibody activity.
f.	Serum-free or low-serum conditions cannot provide sufficient amounts of MAb for some purposes, such as the evaluation of new vaccines against infectious organisms.
g.	Culture methods sometimes yield populations of IgG MAb that are glycosylated at positions different from those harvested from mouse ascites fluid, thereby influencing antigen-binding capacity and important biologic functions.
h.	When hybridoma cells producing MAb are contaminated with infectious agents, such as yeasts or fungi, the cells often must be passed through mice.
i.	Some cell lines that do adapt to tissue-culture conditions become unable to maintain adequate production of MAb

References

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