The use of laboratory animals, especially rodents, as animal models in experimental neoplasia has led to significant increases in the understanding of tumor biology, therapeutics, radio- and immunobiology, carcinogenesis, and other areas of study. In 1975 Kohler and Milstein determined that fusion between myeloma cells (a tumor of bone marrow) and antibody-producing cells produces a hybridoma which secretes large quantities of monoclonal antibody. This discovery has had unmeasurable effects on biology and medicine and led to a Nobel prize. Although the use of animal models in these studies are clearly justifiable, investigators must recognize that animals may experience pain and distress when employed in this manner.

Recommendations provided in this discussion are intended to reduce the pain and distress that can occur when rodents are utilized as animal models for experimental neoplasia or ascites production. It is extremely difficult to establish precise guidelines that apply to all cases and tumor types used in in vivo systems without being severely restrictive. These guidelines should be used as a foundation for designing, writing, and conducting experimental procedures. Appendix I contains highlights of the discussion below and specific recommendations for experimental studies. Deviations from the guidelines should be addressed and justified when submitting your proposals to the Animal Care and Use Committee for review.

Understanding the biology of the tumor or system you intend to employ is critical to the study design and to ensure that pain and distress are minimized for the animal subject. For example, tumors which have a propensity to metastasize may have entirely different effects on the animal as compared to tumors which infiltrate locally. Replication times differ with tumor type and will determine the frequency at which the animals must be observed and the duration of the study. Tumors induced by carcinogens or viruses compound the situation in that these inducing agents may pose additional problems for the animal host. Before animal care and use protocols are submitted, a literature review should be conducted, and other investigators who have used similar (if not identical) experimental methods and/or tumors should be consulted.

The site of tumor implantation is an important consideration regarding potential pain and distress. Sites should be chosen that minimize damage to adjacent normal structures and will not interfere with normal body functions such as ambulation, eating, drinking, defecating, and urinating. Sites involving the special senses such as the eye should be avoided. Intramuscular implantation should not be routinely utilized as distention of muscle with the growing tumor is painful. Subcutaneous or intradermal implantation in the flank is least painful and is preferred.

Tumor burden is one of the most important factors in consideration of animal health and well-being. It is extremely difficult to provide precise guidelines for upper limits of tumor burden as these are dependent upon a number of factors including but not limited to tumor biology, implantation site, and host status. Animals with tumor burdens large enough to interfere with ambulation, eating, drinking, defecating and urinating should be humanely sacrificed. Tumors generally should not exceed 10% of the animal’s body weight, but it is crucial to recognize that an animal may require sacrifice with much smaller tumor burdens.

Tumors may secrete factors which cause severe morbidity independent of tumor size, location, or other aspects of their biology. These animals may become severely debilitated before the tumor has attained appreciable size. Body weight should be monitored when tumors exhibiting these properties are utilized. Study termination will be dependent upon factors other than tumor burden, i.e. percentage of weight loss, etc.

Tumors, like other biologics, may carry viruses or other pathogens which can contaminate animal colonies, infect man and introduce additional experimental variables. All tumors, especially those which have been passed in animals, should be MAP tested to determine their microorganism carrying status. Xenografts of human tumors which are typically grown in immunodeficient hosts may be contaminated with human pathogens and require the institution of appropriate biohazard containment procedures.

Ascitic tumors such as hybridomas require special consideration. These tumors grown in the peritoneal cavity will produce both a solid mass and ascitic fluid. As the volume of the abdominal cavity is limited, severe distention will develop. Distention interferes with a number of physiological systems including but not limited to the respiratory and gastrointestinal systems. Ascitic tumors producing large volumes of fluid can rapidly deplete the animal of essential nutrients such as protein and hasten the cachexia from the tumor. Care must also be taken when ascitic fluid is collected from surviving animals as the rapid removal of a large volume of fluid may cause hypovolemia, renal insufficiency, and edema.

Animals used to produce ascites must be observed at least once daily (including weekends) and often require multiple daily observations. Animals must be tapped before the abdomen becomes severely distended. In general, ascites fluid volume should not exceed 20% of the animal's normal body weight. Because of effects of the tumor and ascites withdrawal described above, animals may not be tapped more than three times with the third tap performed at sacrifice. However, if the animal's condition necessitates it may be necessary to sacrifice the animal earlier than planned. Ascitic fluid collection is performed by placing an 18 or 19 gauge needle into the abdomen of an anesthetized (preferred) or humanly restrained mouse. The abdomen should be swabbed with novaspan solution before the sterile needle is inserted. The animal should be observed carefully for approximately 15 minutes post collection for signs of distress.

Pristane (2,6,10,14 tetramethylpentadecane) is commonly utilized to prime the abdominal cavity of rodents prior to implantation with hybridoma. Pristane is an irritant which prepares the abdominal cavity for seeding with the tumor and interferes with local lymphatic drainage, thus increasing fluid yields. Pristane is toxic at doses only slightly higher than those used to prime mice. Pristane should be administered only once seven days prior to tumor implantation with a maximum dose of 0.5 ml intraperitoneally. Scientific studies in mice indicate that doses as low as 0.1 ml in mice yield ascitic fluid volumes equivalent to the 0.5 ml dose with less animal distress. Because Pristane is an irritant and potential carcinogen it should be handled with gloves to minimize skin contact. Once Pristane is administered, animals should be observed at least once daily for adverse effects. Moribund animals should be humanely sacrificed.
Incomplete Freund's Adjuvant (IFA) has also been successfully utilized as a priming agent for ascites production. Adverse side effects with IFA are less than those observed with Pristane and IFA has the additional advantage that tumors can be implanted into IFA primed mice as soon as 24 hours after its administration. The dosage of IFA utilized is the same as Pristane.

Studies utilizing mice in experimental neoplasia and ascites production must have precise end points. Death as an end point, except in unusual circumstances, is not considered suitable. When designing experiments and preparing animal care and use protocols end points should be developed and precise guidelines for animal sacrifice should be provided. Whether animal sacrifice is dependent on tumor size or time period post implantation, animals must be observed daily by the investigator to determine that animal morbidity and distress are minimized. Attention should be given to the animal's overall appearance, weight, respiratory rate and pattern, color, fecal and urinary output, size of the tumor, etc. Animals may require sacrifice before established guidelines if they appear moribund. The Animal Resources veterinary staff should be contacted when moribund or distressed animals are noted; appropriate supportive and/or analgesic therapy may allow the experiment to continue humanely.

REFERENCES


APPENDIX I

HIGHLIGHTS

of

Guidelines for the Use of Rodents in Experimental Neoplasia and Acites Production

A. In vivo experimental neoplasia

1. All transplantable tumors should be assayed for contamination with adventitious murine viruses.
2. Tumor implantation sites should be chosen to minimize damage to adjacent normal structures. Sites involving the special senses should be avoided; intramuscular implantation should be avoided as distention of muscle is considered painful. Subcutaneous or intradermal implantation in the flank is considered acceptable.
3. Tumors generally should not exceed 10% of the body weight of the animal. However, when utilizing particular sites (i.e., intraperitoneal implantation), growth should be severely restricted.
4. Tumors should not interfere with normal bodily functions (i.e., ambulation, eating, drinking, defecating, and urinating).
5. Animals should be humanely sacrificed prior to tumor ulceration.
6. Animals should be examined at least daily after tumor implantation.
7. End points of experimental neoplasia should be determined and specified in the experimental protocol. Death is generally considered an acceptable end point unless clear justification is provided.

B. Acites production (rodents)

1. Rodents should be primed once with a maximum of 0.5 ml Pristane. Preferably a volume of 0.1 - 0.2 ml should be utilized. Incomplete Freund's Adjuvant (IFA) may be substituted for Pristane.
2. Rodents should be examined at least daily once they have been primed with Pristane or IFA and the procedures for myeloma implantation have begun.
3. Ascitic fluid should be tapped prior to gross abdominal distention and distress.
4. Rodents should not have ascites fluid harvested more than three times; an 18-gauge or higher gauge needle should be used. The third harvest should be performed after sacrifice. Animals which are in distress (i.e., cachexic, not eating, or with increased respiratory rates) should be sacrificed immediately. In general, ascites volume should not exceed 20% of normal body weight.
5. End points should be determined and specified in the experimental protocol. Death is not an acceptable end point with ascites production.

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