Chapter XXI: Animal Models: ATm/ATr, Fanconi Pathway Proteins, and Scaffold for Nuclear DNA Repair

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Animal models for use in Radiation Biology

Appropriate animal models for studying the effects of ionizing irradiation on total body effects, and each of multiple organs and organ systems have been described in detail over the past decades. This chapter of the web-based textbook will describe animal models commonly used for Radiobiology research in each of these areas. Readers should refer to each of the individual chapters in this book on organ specific and organ system related radiation biologic events. Furthermore, since planning experiments with the "appropriate animal model" should also reference the chapter on FDA regulations and the "animal rule" for validating data in experimental models with respect to translation of research findings to human experimentation and human use.

After discovery of radioisotopes (elements that emit ionizing irradiation: gamma rays, beta particles, and alpha particles), and the discovery of the cathode ray tube (x-rays) at the turn of the 20^{th} Century, it became obvious that prolonged irradiation exposure resulted in significant deleterious effects on humans including research physicists, chemists, and physicians, who used cathode ray tubes for x-ray fluoroscopy. Clinical observations indicated that total dose; the dose rate of exposure, as well as volume of tissue or organs exposed, determined the severity of injury.

The first scientists and physicians to use x-rays also suffered radiation late effects, notably, induction of leukemia and solid tumors. Animal models for understanding the mechanisms of radiation injury, and for developing treatments, soon became available. In most cases, the expense of animal housing and maintenance directed investigators to the use of small animals, mice and rats. However, the appropriateness of data obtained in rodent animal models could not be easily translated to humans for all indications, and larger mammalian species soon became a subject of research including: pigs, dogs, and non-human primates.

Each experimental system will be described in the present chapter in sequence, focusing the reader on the appropriateness of each model for specific studies.

Total Body Irradiation (TBI)

Radiation exposure of outbred mice and rats first led to the discovery of specific effects on organs and organ systems including the bone marrow and intestine. Depending on total body dose, outbred rodents demonstrated a dose dependent reduction in bone marrow cellularity, as observed under the microscope, and also shortening of the intestinal villi and death of cells in the crypts (base of the villi) of the small intestine. The availability of radiolabeled nuclides for uptake in the intestine and bone marrow facilitated an understanding of irradiation effects on dividing cells. These data from rodent TBI exposure experiments correlated well with <u>in vitro</u> studies and specifically, the susceptibility of dividing cells (compared to quiescent cells) to irradiation.

Variation between individual animal responses was also observed in early experiments. The development of inbred mouse and rat strains greatly enhanced the ability of scientists to obtain reliable and reproducible data (1). Experiments with outbred Swiss mice were reproduced using

genetically inbred mouse strains, and TBI experiments revealed genetic variation between inbred strains (2). C57BL/6 mice were observed to be relatively radioresistant compared to the radiosensitive C3H/He and Balb/c mice (3). A broad spectrum of inbred mouse strains became available in the 1950s owing to work of Jacob Furth, and led to the establishment of specific laboratories and facilities for breeding inbred mice, notably the Jackson Laboratories in Bar Harbor, Maine (4). Immunology of the irradiation response, which determined kinetics of organ toxicity, and the speed of return of both hematopoiesis and lymphopoiesis (appropriate immune recovery) allowing response to pathogens, as a discipline, was facilitated by use of inbred mouse strains. Mice with specific immunologic defects and biomarkers for subsets of lymphocytes, allowed radiobiologists to study the irradiation effects on immunity. Rodent models became widely used for understanding the effects of irradiation on subsets of dividing cells within the bone marrow and intestine.

In the 1960s and 1970s, work from the Netherlands, including that of Ploemecher and colleagues (5) showed the relative radioresistance of true totipotential hematopoietic stem cells and paved the way for research in subsequent decades showing the importance of the bone marrow microenvironment in holding stem cells in quiescence, thus, protecting them from cell division and mitotic death.

Specific genes and clusters of genes involved in the radiation response were defined in inbred mouse strains. Beginning in the late 1970s, with the capacity for breeding homologous recombinant deletion (knockout) mice and (transgenic) mice carrying transgenes inserted into DNA for overexpression of certain gene products, new studies were possible. The use of embryonic stem cells to create such knockout and transgenic mice led to studies of differentiation of the most primitive cells in vitro and the different radiosensitivities of specific phenotypic lineages (6). All of these studies were carried out in rat and mouse models.

Total body irradiation studies that were appropriate for elucidating the human radiation response were first developed during World War II with the Manhattan Project and research into nuclear fission required for development of the Atomic Bomb (7). Large animal models were used to simulate human exposure to high dose rate gamma radiation such as experienced during the explosion of a fission bomb. Studies were carried out with pigs, sheep, and non-human primates to simulate the effects of ionizing irradiation burns, thermal burns, and organ injury. These were the first studies of combined injury looking at thermal burn, overpressure/concussion injury, and ionizing irradiation. Large animal studies formed the basis for protocols using each specie for testing specific radiation countermeasures.

Because of the expense of large animals and animal housing, current studies of late effects in radiation research have been primarily concentrated on rats and mice. The development of inbred, knockout, and transgenic mouse strains facilitated studies of leukemogenesis and carcinogenesis. Specific mouse strains with overexpression of p53, specific oncogenes including C-Myc, N-RAS, and others allowed studies of the kinetics of irradiation-induced late effects (8). These publications have answered many questions, but have created others. A major and fertile area for research is the understanding of how exposure to a relatively low dose of irradiation can make specific mouse strains resistant to a subsequent leukemogenic dose of radiation (9). These data suggested that the low dose irradiation response primes the upregulation of stress response

genes, and thus, "prepares" the animal for relative resistance to a leukemogenic dose of radiation to follow. The molecular mechanism of these changes is the subject of intense investigation, and of importance to the development of radiation countermeasures. In particular, mouse strains exposed to the "priming" or protective" low dose irradiation can now be studied for the alterations in the molecular mechanism of irradiation response in its specific tissues and cell lines explanted to tissue culture.

Specific radiation sensitive and radiation resistant mouse strains.

Molecular biology studies have revealed changes that are correlated to specific human diseases. These studies led to a discovery of a wide array of genes associated with radiosensitivity and others with radioresistance. Readers should consult the chapter on the molecular mechanism of irradiation damage repair to DNA (Bakkenist and Greenberger) to understand how each of several genes associated with DNA repair led to the generation of research of mouse strains with overexpression or deletion of specific gene products. The first molecule known to arrive at the site of a DNA double strand breaks is the ataxia telangiectasia (AT) mutant (ATM) protein (10). This protein is that associated with the human disease AT in which patients are sensitive to ionizing irradiation. Studies of ATM inbred mice have led to the understanding of organ specific radiation damage and specific cell phenotype responses to irradiation. A related (11) ATR gene has been shown to be involved in chromosome instability in radiation late effects also has been studied in mice genetically altered to express this protein. Total body irradiation studies on these animals is a fertile ground for research. Similar knockout and transgenic mice associated with Bloom's Syndrome (12), and the sections associated with the Fanconi Anemia pathway (13) are now available for such TBI studies and are profoundly interesting.

Fanconi Anemia represents an inherited disease first described in the early 20th Century and associated with new short stature, absent thumbs, café Au Lait spots, and other abnormalities (13). These children develop anemia, and are also found to be sensitive to ionizing irradiation. Over the past 5 decades, the FA pathway has been shown to represent a group of 23 different proteins, which form a scaffold at the site of DNA double strand breaks, and arrive at this location shortly after the first arrival of ATM. Availability of FA protein knockout and transgenic overexpressing mice has facilitated many studies on the radiobiology of total body irradiation.

In contrast, mouse strains genetically resistant to ionizing irradiation include animals that are unresponsive to the irradiation-induced cytokine TGF-β (14). Discovery of the signaling pathway for TGF-β binding to its receptor and activation of a series of proteins referred to as SMAD series of proteins by Joan Massague, led to oxidation of the TGF-β signaling pathway. Work by Flanders, et al. (14) demonstrated that SMAD3 knockout mice were resistant to irradiation-induced fibrosis (late effect), but subsequent studies showed that their bone marrow was also radioresistant and cell lines derived from these animals showed radioresistance in vitro (15). A large series of genetically altered mice both deleted for expression of gene products and overexpressing are now available for understanding many subtle effects of ionizing irradiation. Other radioresistant mice include those overexpressing antioxidant enzymes such as Manganese Superoxide Dismutase (SOD2), and homologous recombinant negative (knockout) mice for

MnSOD are radiosensitive. Conditional knockout or knock-in mice are also available for many of these irradiation related proteins (16-17).

The CRE-recombinase transgene allowed the development of animals that could respond to the addition of an inducer protein on another chromosome (LOX) produced by a specific drug sensitive promoter such as Doxycycline or Tamoxifen. Treatment of such CRE/LOX mice with these inducer proteins facilitated the turn-on or shut-off of a specific gene. While some of these conditional knockout or knock-in mice show "leakiness" of the promoter and a complete turn-on or shut-off of the gene product, better technologies are being developed to make these conditional animals more tightly regulated (17). The area of conditional knockout mice is a great area of research for new radiobiology investigators.

Timing of the appearance of a specific gene product can be manipulated with conditional knockout mice and may be found to be critical for gestation and normal development in utero. Eliminating the gene in the embryo may be problematic. For example, animals missing TGF-β signaling are either dead in utero or demonstrate neonatal death. Studies with knockout mice are very important not only for those used in current research, but also for those in which the genetic alteration is lethal. Understanding the mechanism of lethality in utero can be very important for research in radiation effects in the developing embryo. Other chapters in this book describe the sensitivity of early stage embryos (analogous to the first trimester in humans) relative radioresistance of a late stage developing embryo. The mechanism of radiation killing of primitive cells in the developing embryo may be quite different from that of rapidly dividing cell populations in the adult bone marrow and intestine. This is another important area for research. Availability of embryonic stem cells, inducible pluripotential stem cells, and other new technologies allows radiobiologic research in these critical areas. This research is made possible by the availability of transgenic and knockout mice.

Total body irradiation and bone marrow transplantation.

This area will be covered in the chapter by John Chute, and emphasizes the importance or animal models for bone marrow transplantation. Radiobiology of stem cell and the microenvironment is critical.

Thoracic Irradiation

Rodent animal studies are valuable for elucidation of the effects of ionizing irradiation on the lungs, heart and thoracic spinal cord. These have utilized a technique of thoracic irradiation (18). Under these conditions, the abdomen and lower extremities are shielded, either by constructing a jig that inserts into a Cesium gamma cell animal irradiation unit to protect the lower body, or use a clinical orthovoltage x-ray machine or linear accelerator to block the abdomen and lower extremities. It is important for investigators to differentiate between upper body irradiation and thoracic irradiation. In the former situation, the head and upper extremities of the rodent may be included in the beam. In the latter situation, the head and neck are shielded. This difference should be well described in the Materials and Methods sections of any manuscript, and for experimental planning, investigators should be aware of the profound difference between these techniques. There is a significant volume of bone marrow in cervical spine and skull of rodents.

Furthermore, irradiation delivered to the oral cavity and oropharynx produces significant side effects, which can complicate the study of thoracic irradiation. Finally, irradiation of the brain produces significant effects on the heart and lung.

Thoracic irradiation is carried out with the head and neck, and the lower body shielded. Thoracic irradiation is dose dependent, and dose rate dependent with respect to both acute and late effects. Some animal model systems are well established and investigators are advised to use the C57BL/6 strain, which readily develops radiation fibrosis, but not acute pneumonitis. The acute effect is not correlated with induction of late radiation fibrosis at 100 – 120 days after 19 Gy to the thoracic volume (19). In contrast, C3H/He mice develop acute radiation pneumonitis and do not show detectable late radiation fibrosis. The molecular mechanism for these differences has been elucidated (3, 19). However, the genetic regulation of the difference between these mouse strains is not known. There is significant clinical correlation of the genetic differences between these mouse strains with the appearance of radiation fibrosis in some patients treated for lung cancer, esophagus cancer, or thoracic lymphoma. Understanding the molecular control of radiation pulmonary fibrosis is a major goal of current radiobiology research.

The thoracic radiation paradigm when applied to rodents, as described above, can be a fertile system by which to test radiation dose modifying agents. Drugs that treat fibrosis or prevent fibrosis are much anticipated, and research into this area, particularly small molecule radiation dose modifiers is a major goal of radiobiology. Gene therapy techniques have been utilized to prevent radiation fibrosis (20). A major focus in the modern era is that associated with small molecule mimics of gene therapy products, such as the use of small molecule GS-nitroxides to mimic Manganese Superoxide Dismutase gene therapy (20, 23).

Thoracic irradiation models include ones in which there is intratracheal injection, systemic intravenous injection, and recently, a technique of inhalation gene therapy (21). These routes of administration of radiation protector and mitigator drugs can be utilized.

Microbeam irradiation techniques and micro-irradiators are available to treat small portions of the mouse or rat lung. Large animal models for study of thoracic irradiation effects are more expensive and difficult to manage, but tests of countermeasures against radiation lung damage is being confirmed in current studies with non-human primates (22).

Radiation effects on the heart include studies in rodents demonstrating radiation fibrosis, damage to the cardiac vessels, and most recently, radiation induced arrhythmias. Studies in non-human primates have shown increase in thoracic irradiation-induced myocardial infarction, coronary artery strictures, and these studies have recently been published (22).

Head and Neck Irradiation

Elegant animal models to produce radiation mucositis, which is a common side effect in the radiotherapy of head and neck cancers, have been described. These experiments require shielding of the entire rodent body except the head and neck region. Depending on the mouse strain, single fraction doses of 30 Gy to the head and neck can reproduce the mucositis seen in patients receiving 60 Gy over 6 weeks in fractionated irradiation. Fractionated radiation

protocols are available to simulate fractionated irradiation effects in humans and have included a paradigm of 4 Gy daily for four days (23-25). Fractionation experiments allow administration of radiation protector or mitigator agents to assess the effect on acute radiation damage. Late radiation fibrosis in the oral cavity and head and neck has also been described in rodent models of single fraction or fractionated irradiation (23-25).

For experiments on head and neck irradiation, it is critical for the investigator to have a physicist carry out precise thermoluminescent dosimetry or other ionization chamber measurements of the internal scatter dose to the chest and abdomen. Precise culmination of the radiation beam using a linear accelerator or gamma cell irradiator should be confirmed. Microbeam irradiators to treat small areas in a rodent model make this technique easier, but still require medical physicists collaboration to confirm the dose and scatter dose outside the target volume.

There are numerous publication, which accurately detail the methodologies for studying radiation effects on the oral cavity and oropharynx. Assays for tissue damage including mucositis and percent ulceration of the tongue and oral mucosa have been published (23-25). Very important to the study of head and neck irradiation is the salivary gland function. There are histopathologic data describing radiation injury to salivary glands, but also assays for cannulation of the parotid gland duct, and measurement of saliva production. These assays are very well described (26). Minor salivary glands in the mouse include those sublingual and axillary. The saliva component to all forms of radiation damage to the head and neck is critical. Methodologies by which to improve radiation response of orthotopic tumors (Tumors injected and placed in the oral cavity tissues for measurement of tumor size and radiation reduction in size) have been published. The most interesting areas of research in head and neck radiotherapy, using animal models, are those describing differences between radiation protector drugs, which can spare radiotherapy killing of tumor, while preserving normal tissue anatomy and function, and those studies of radiation sensitizers for tumors (26). All of these studies are very well carried out with orthotopic tumor models in the head and neck region.

Abdominal Irradiation

The GI syndrome is that associated with irradiation doses above the total body irradiation doses that can be mitigated by bone marrow transplantation. For these studies, the thoracic region including the head and neck is shielded, as well as, lower extremities (29). The abdomen from the diaphragm down to the pubic synthesis includes all the small and large intestine, and also the stomach. With the availability of microbeam irradiators for animal irradiation studies, the stomach can be spared, as well as, pelvic organs. However, irradiation damage to the GI tract is primarily measured by damage to the ilium. The stomach, duodenum, and jejunum are less damaged by abdominal irradiation doses at multiple levels than is the ilium. The colon (large intestine) is largely radioresistant. Assays for abdominal irradiation damage were developed originally by Rodney Withers and described in detail, as the “gut colony assay” (28). Animals (rodents) are given total abdominal irradiation, and then at serial time points pulsed with a radiolabeled nucleotide that can be taken up in dividing cells, principally, in the crypt of the intestine. This assay is carried out by measuring the length of the villus, as an index of irradiation damage, but also uptake in cells of the crypt of radionuclide. The crypt sites are traditionally considered those areas, where gut stem cells reside.

The field of Radiation Biology of the intestine has greatly changed by demonstration of the transdifferentiation capacity of intestinal cells, not only in the crypt, but lining the villus. Migration from the walls of the villus into the crypt has been shown to occur in both directions. Furthermore, damage to true stem cells in the intestine can be quantitated by measurement of LY5 (27) and other biomarkers for the intestinal stem cell. These biomarkers are clearly different from those of the bone marrow stem cell. While the bone marrow and intestinal stem cells are both critical for the acute radiation response and are the target for developing radiation mitigators, there are multiple cells in the intestine (as in the bone marrow), which contribute to the survival of stem cells. Goblet cells in the intestinal villi produce mucin, which is a major component of a barrier between the intestine and the gut microbiome. Damage to the barrier function in the intestine is a major contribution to the GI syndrome, and death from damage to the intestine. The hematopoietic syndrome and GI syndromes overlap. Studies by Travis, et al. (29) and Krause, et al. (30) demonstrated that cells of bone marrow origin repopulate the intestine after abdominal irradiation. The first cells to arrive in the intestine after irradiation damage and at the onset of repair are those of the immune system. The intestinal immune system is distinct and extremely important in both regeneration of the damaged epithelial cells, but also in defense against bacterial pathogens in the intestine.

The importance of the microbiome in radiobiology of the intestine is of great interest. Microbiome differences between different rodent animal suppliers may explain the difference in radiosensitivity to total body or abdominal irradiation between experiments and at different times of the year. Recent publications have stressed the importance of three components of intestinal irradiation injury: the irradiation damage to the intestinal cells themselves, the change in the intestinal microbiome after irradiation, and the change in bile salt metabolism in the irradiated intestine (metabolomics) (31). Investigators seeking to enter the area of intestinal radiobiology should familiarize themselves with the literature on the microbiome, intestinal barrier, as well as, the multiple cell phenotypes in the intestine.

Readers should consult the chapter on overlapping pathways of death syndromes following radiation to understand the importance of not only apoptosis, but necroptosis and ferroptosis in irradiation damage to the intestine. A recent publication with lung radiobiology (soon to be confirmed with studies of the intestine after irradiation) demonstrates the ability of specific strains of bacteria to “hijack” cell death mechanisms in epithelial cells. In a recent publication (32), pseudomonas aeruginosa, which gains growth advantage after barrier breakdown of the immune defense in the lung, in the case of long-term ventilator patients and cystic fibrosis patients, who have defective sodium pump mechanics in pulmonary epithelium, demonstrate that this bacteria can induce ferroptosis in pulmonary epithelial cells causing further damage and entry of other bacterial and viral pathogens. Similar events may be occurring in the intestine. There will undoubtedly be other examples of microbiome changes in the irradiated intestine, the role of diet, the potential effect of probiotics as radiation mitigators, and, of course, the effects of single and multiple small molecule radiation measures. Intestinal radiobiology is a primary for future research.

Radiation of bone, muscle, and joints

There has been much research on the effects of irradiation on the extremities. Herman Suit first described the TCD_{50}, which was a tumor control dose (33), experiments in the mouse limb, in which orthotopic tumors were placed, and then irradiated using specific single fraction or fractionated irradiation. During these studies, limb retraction was observed. Studies in the effects of irradiation on muscles, joints, and bone have been described in multiple rodent species. Gorodetsky, et al. demonstrated skin irradiation effects, but reproduction of these data in muscle and bone have been carried out in C57BL/6J mice (34). Muscle irradiation, particularly, to doses of 30 Gy in the case of the C57BL/6J mouse induces muscle fibrosis. This is measured by decrease in limb excursion (range of motion). The use of radiation mitigators including injection of adipocyte stem cells can ameliorate the reduction in limb excursion by radiation.

The effects of irradiation on bone growth have been described in recent years (35). In studies with C57BL/6J mice, unicortical (one site of the conical bone) bone wound was established in the proximal tibia by using a dremel drill, bone healing was observed in 21 days. Irradiation to that site significantly decreased bone wound healing. Studies with radiation mitigators, JP4-039 (23), MMS350 (36) demonstrated reduction in the delay of bone wound healing by irradiation. This model system has been widely accepted, and is less traumatic to animals than the previous fracture model used in rabbits.

Bone wound healing is delayed by irradiation. The pathophysiology is poorly understood, and this is a great area of research. In comparison of C57BL/6J mice with SAMP6 (senescence associated delay bone healing and osteoporosis) compared to nu/nu (immunodeficient mice), the pattern of bone wound healing was seen to be different between these mouse strains (37). After unicortical bone wound or fracture, a clot is formed, very much as is the case in other wounds. The clearance of the blood clot by migration into the site of the wound of endothelial progenitor cells is detected in all models. Endothelial cells lead to migration of osteoblast progenitors, which differentiate from the bone marrow stromal cells. Osteoblasts, then, begin the process of creating primary osteoid to begin the healing process, then bone remodeling by osteoclast interactions with osteoblasts is a standard phenomenon in all fracture bone wound healing (37). The effect of irradiation on these different cell phenotypes has not been studied, nor has the targeting effect of radiation protectors or mitigators on these different cell populations. The methodologies for all of these studies are published, and the investigators are encouraged to continue research in this area. In the field of radiation countermeasures, combined injury of bone fracture along with other traumatic injuries and irradiation is a subject of intense investigation for the development of new radiation mitigators. Investigators in Dept. of Orthopedic Surgery, Rheumatology, as well as, Basic Cell Biology are encouraged to continue studies in the effects of irradiation on fracture healing and the use of radiation mitigators to ameliorate the trauma.

Radiation Effects on the skin

Beta-irradiation burns (electron beam irradiation) is well known in both clinical radiotherapy, and from studies of the Hydrogen Bomb fall-out casualties in the Marshalles Islanders (38).

Isotopes including radioiodine and cesium, when covering the skin can produce significant beta-irradiation burns. The readers should consult the chapter on triage of radiation terrorism

casualties or management of patients from nuclear reactor accidents to understand how to manage and minimize beta-irradiation burns.

Treatment of beta-irradiation burns has been advanced with the development of topical (locally applied to the skin) formulations containing GS-nitroxide radiation mitigators (39). Other forms of treatment for irradiation burns have been similar to those used for thermal burns (reader should consult the chapter on thermal burns by Jones). Skin irradiation damage is modulated by the application of radiation mitigators. Late radiation fibrosis of the skin is a major complication of clinical radiotherapy to head and neck cancer patients, and in some patients treated to the chest wall for breast cancer, who did not benefit from the advent of intensity modulated radiotherapy (IMRT), which reduces the high dose area in the medial and lateral tangent entry fields. Long-term follow-up of breast cancer patients, has demonstrated significant radiation fibrosis in these areas, which results from migration into the irradiated tissue of fibroblast progenitors of bone marrow origin, but also from proliferation of fibroblastic-scar forming cells from adjacent tissue into the irradiated area. The development of radiation mitigator and treatments to prevent or resolve radiation-induced scar formation has focused in the same radiation mitigators currently studied for the acute radiation effect on the hematopoietic syndrome and GI syndrome.

Animal models for irradiation to the brain and spinal cord.

Rat and mouse models for brain irradiation have been well described (40-41) and include both pathology and pathophysiologic measurements of brain function. There have been many successful applications of assays for neurocognitive function in rodents. The Morris Water Maze, Novel Object Recognition, Fear Conditioning, and other behavioral assays for assessing irradiation damage to the brain have been well described (40-41). Dose response curves of single fraction and fractionated irradiation to the brain have revealed quantitative information on pathologic changes in the brain, particularly, the sensitivity to the hippocampus, and decrease in production of migration of neural stem cells in the cerebral aquaduct systems, and alterations in glial cell proliferation. The work of John Fike (40) has demonstrated the importance of superoxide dismutase (SOD) in brain recovery from irradiation in mouse and rat models. Elegant techniques have been published for histopathology to the brain, pathophysiology, and functional assays for stress response gene transcription and protein production.

Spinal cord irradiation damage has been shown to be volume, dose, and fraction size dependent. The work Van Der Kogel, et al. (42) and colleagues in rodent models showed the importance of total volume irradiated, but also a high dose section of spinal cord irradiated by itself produced less radiation damage than if that high dose area was bordered by two other areas receiving a lower dose. These studies suggested that migration into the high dose irradiated site of cells from the spinal cord blood circulation, as well as, CSF (cerebrospinal fluid) circulation could be involved in the response to irradiation damage. Spinal cord irradiation biology is another extremely fertile area for research. Animal models of spinal cord damage are well described. Diseases of the spinal cord including ones in which anterior horn cell motor neurons are depleted by the disease process (Amyotrophic Lateral Sclerosis-ALS) (43) allow studies to the interaction of irradiation with neurodegenerative diseases. Animal models of rodent systems are widely available for these studies.

Conclusions

New investigators seeking to carry out radiation biology studies with animal models should consult guidelines for the appropriate model. Each situation, in most cases, is a rodent (rat or mouse) model for radiobiology in these areas. Using other models including small animals such as hamsters, gerbils, and even rabbit or ferret, can be appropriate for a specific indication. For example, the ferret is used in studies of continuous low dose irradiation by NASA investigators looking at effects of cosmic irradiation inducing vomiting (44). Nausea and vomiting as a side effect of irradiation is rare in animal models.

The expense of radiobiology studies using large animals including pigs, dogs, and non-human primates must first be evaluated with respect to the goals of the experiments and the hypothesis. Most institutional animal care and utilization committees seek to prompt investigators to use the lowest of the animal species to obtain an answer to a specific question. Investigators should consult the FDA guidelines, and specifically, the chapter by David Cassatt to first understand how the approach to new radiobiology experiments should factor in (at the earliest stage) the appropriate animal model.

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