Chapter VIII. Acute Radiation Effects: Total Body, Subtotal, Species Differences:

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Introduction:

The radiobiology of total body irradiation incorporates physics, radiation biology, and genetic factors, and above all, is dependent upon the species and age of the study population. This chapter will review general principles of the study of total body irradiation and evaluate each of the critical concepts that are necessary to consider for any planned experiments. Necessary to any studies of total body irradiation is an understanding of the clinical concepts of radiation beam quality, dose rate, and the homogeneity of dose experienced by the test population. Readers should consult Chapter V on the origins of life on the planet, and the concept of continuous adaptation to ionizing irradiation during the evolution of species. Also, it is important for investigators wishing to study total body irradiation is the literature on the best and most appropriate animal model.

Total body irradiation (TBI) has been used for decades to prepare human bone marrow transplant patients for engraftment by donor bone marrow stem cells innoculation. Therefore, a rich literature on clinical TBI radiotherapy is also available for study. The most reported animal models for total body irradiation (TBI) include studies carried out in both out-bred dogs (the pioneering work of E. Donell Thomas of the Fred Hutchison Cancer Research Institute), and many studies in genetically in-bred mouse and rat strains. Studies in the effects of TBI on non-human primates are reported in the chapter in this book by Drs. Mark Cline and John Olson.

The Important Role of Radiation Physics in Study of Total Body Irradiation

Radiation dosimetry is critically important in analyzing total body irradiation events (exposure of humans to TBI or animal model experiments), and the concepts involved in radiation physics all apply in this discipline: dose rate, radiation beam quality (composition of photons, neutrons, protons, and other high linear energy transfer particles such as Carbon14), as well as determining whether parts of the body of subjects received lower or higher doses. Dose rate is critically important in understanding TBI effects as higher dose rate is more lethal for any given dose. There are many excellent textbooks, which describe and summarize the data on the role of species and genetics on sensitivity to total body irradiation. It is not the purpose of this chapter to review the literature on TBI, but rather to serve as a summary of the general principles and provide references for new investigators wishing to study TBI. Important in such studies is the choice of animal species, strain (mice and rats), and need for multiple control groups in any such studies.

The radiobiology of total body irradiation is different from that of radiation effects on specific organs. Several general principles apply to studies of TBI.

Early bone marrow transplantation studies in the 1960s by E. Donell Thomas, George Santos, and others led to the discovery of circulating bone marrow hematopoietic stem cells. In total body irradiated dogs and mice, it was observed that leaving one limb out of the radiation beam resulted in reconstitution of the bone marrow from hematopoietic stem cells that resided in the non-irradiated microenvironment (1). These studies established several important concepts: 1) There was a radiation dose above which bone marrow transplantation could not rescue animals from lethality, and this dose defined the upper limit of the hematopoietic syndrome; 2)

Circulating hematopoietic stem cells from shielded volumes of marrow as low as 10% of the total bone marrow could completely reconstitute hematopoiesis in irradiated animal; 3) Doses above that which could be rescued by bone marrow transplantation resulted in death from a separate syndrome associated with gastrointestinal symptoms and death of stem cells in the small intestinal crypt – GI syndrome (2). A vast literature on total body irradiation of genetically inbred mouse strains led to understanding the importance of genetics in tolerance to total body irradiation. There is a steep dose response curve leading to lethality from total body irradiation in mice. Genetically, radioresistant mice (C57BL/6) compared to radiosensitive mice (BALB/c) both demonstrate a resilience/recovery from total body irradiation at doses below a specific threshold. The dose response curve of TBI in mice is similar to the shoulder on the radiation survival curve of clonogenic cells in vitro (See the chapter on Methods in Radiobiology). At TBI doses above which animals begin to die, there is a very steep dose response curve leading rapidly to the $LD_{100/30}$ dose of radiation. One hundred percent of animals dead at 30 days. This concept of lethal dose (LD) at 30 days led to the main parameter for understanding the hematopoietic syndrome. The $LD_{50/30}$ (lethal dose for 50% of mice at 30 days) is observed on the TBI radiation dose response curve and defines the radiosensitivity of one mouse strain and radioresistance of another (Balb/c compared to C57BL/6). However, these kinds of experiments cannot be carried out unless the TBI radiation beam is precisely calibrated. Readers should consult the **chapter by Ke Sheng, Ph.D.** on Radiation Physics to be certain that radiation beam quality and stability are defined before any experiments are carried out. This point cannot be over emphasized. The dose rate and beam quality (if 100% photons – x-ray or gamma rays then what energies? Do energies fluctuate?) of any delivery system must be kept steady over time if any TBI studies are to be carried out.

For example, a total body irradiation dose of 9.25 Gy is the $LD_{50/30}$ for C57BL/6 mice and the $LD_{50/30}$ is 8.0 Gy for BALB/c mice, in one animal facility using one particular radiation source. This data was obtained at the University of Pittsburgh Cancer Institute, using a $Cesium^{137}$ gamma cell Mark IV irradiator delivering radiation at 70 cGy per minute. The data and results change markedly if the dose rate is increased. Removing the filter from this particular gamma irradiator increases the dose rate to 350 cGy per minute and may change the $LD_{50/30}$ (more lethal at each dose with higher dose rate) for both mouse strains. However, the conditions of animals in the irradiator also are different and must be considered. The animals are in the immobilizer for shorter time if dose-rate is higher. The increased dose rate causes rapid DNA double strand breaks in critical cells of the bone marrow and intestine, which are the target organs for death from total body irradiation (The hematopoietic syndrome is recoverable by bone marrow transplantation indicating that under these conditions, the intestine tolerates the radiation dose.). However, damage is sustained by both these critical organs (marrow and intestine), as well as other organs. A higher dose rate allows less time for repair, and cellular adaptation within tissues, organs, and organ systems is less, thus increasing the lethality from a given radiation dose. However, the animals are immobilized for a shorter time to sustain each dose, and they will experience less stress. Stress plus TBI is more lethal than TBI alone (See chapter on combined injury.). Both issues should be considered for TBI protocols.

Radiobiology of TBI

Standardization of the physics of irradiation beam, once achieved, allows investigators to determine the role of biology and genetics in TBI sensitivity. Inbred mouse strains provided valuable information in the role of genetics in radiosensitivity. A radiation-resistant mouse strain, C57BL/6, and radiosensitive mouse strain, C3H/HeJ, were studied in elegant genetic experiments by Elizabeth Travis, and using F1, F2, and back-cross generations, a genetic locus for radioresistance was identified and associated with one specific site on one chromosome. Multiple genes at this site were implicated in the radioresistance of one strain and sensitivity of another.

Specific genes are known to influence radiosensitivity. The availability of transgenic and homologous recombinant negative (knockout) mouse strains facilitated these studies. For example, the ataxia telangiectasia mutation knockout mice (ATM-/-) are markedly radiosensitive compared to the control mice on this genetic background strain. See the chapter on DNA repair pathways. Similarly, knockout mice for one or more of the genes in the Fanconi Anemia pathway (Fancd2-/-, Fancg-/-, Fanca-/-) have been shown to be also radiosensitive (17). Studies relate to the $LD_{50/30}$ acute effects of total body irradiation.

TBI also has other effects, which can be studied when animals are exposed to doses below the $LD_{50/30}$. These experiments relate to radiation late effects. Total body irradiation late effects are a distinct category and should be considered separately. Prominent in the late effects of TBI (also related to genetics and the roles of specific gene products) are the phenomena of irradiation-induced aging and irradiation-induced fibrosis. Genetically inbred mice have been shown to age more rapidly following total body irradiation compared to non-irradiated controls (28-29). Aging is associated with easily quantifiable parameters including hair greying, arthritis and joint instability, weight loss, and early death. These parameters also change based on the genetics of the mouse strain being studied. Homologous recombinant negative (knockout) mice deficient in one of the critical gene products involved in the TGF-β pathway (SMAD3-/- mice) are markedly resistant to a prominent late effect of irradiation, namely radiation fibrosis (20). There is often correlation between radiation resistance of cells in culture and resistance to total body irradiation late effects. In the SMAD3-/- mouse strain, both hematopoietic and bone marrow stromal cells are radioresistant, and the animals resist radiation-induced fibrosis of skin, lung, and other organs. The effect of TGF-β signaling in both response of cells in culture and animals in total body irradiation is well documented in this strain, where radioresistance is uniform.

Correlation of cell line radioresistance with resistance to fibrosis is not always clear cut. While Fancd2-/- mice (both C57BL/6 and 129/Sv background) are radiosensitive, cells in culture do not show uniform radiosensitivity. In these mouse strains, fresh marrow hematopoietic colony forming cells, and clonal hematopoietic cell lines derived from marrow culture are radioresistant; however, bone marrow stromal cells, which support the hematopoietic microenvironment in both of these mouse strains are radiosensitive (17). The data suggest that the animal is radiosensitive, because of an indirect effect of the cells in the microenvironment on hematopoietic stem cells. In both genetically inbred mouse strains and outbred strains, there are other possible explanations for the differing radiosensitivity of different cell populations. One radiosensitive population may release inflammatory cytokines or altered cell surface molecules after irradiation exposure, such that a second population is pushed along one or more death pathways (apoptosis, necroptosis,

ferroptosis, lysosomal necrosis). For a study of these death pathways, one should consult the chapter on Cell Death Pathways Induced by Irradiation. The influence of cytokines or adhesion molecules released by radiosensitive cells, and associated cell populations may adapt to TBI by upregulation of antioxidant stores, induction of genes for enzymes important in the radioresistance pathway, and that cell population if separated from the radiosensitive supportive microenvironment, may in fact show radioresistance. This data may be the explanation of the results with Fancd2-/- mice in which the entire animal is radiosensitive, but a separate cell population of hematopoietic cells is paradoxically radioresistant if isolated and studied separately ex vivo in cell culture.

Species Differences in Total Body Irradiation Response

The condition of a particular species in the phylogenetic tree in evolutionary biology does not determine relative radioresistance to TBI. Studies in bacteria and yeast demonstrate the role of many genes conferring radiosensitivity in vitro and in vivo. With respect to intestinal bacteria (microbiome), total body irradiated mice have been shown to have an altered microbiome with respect to the numbers and diversity of strains of bacteria in the intestine at variable times after irradiation. These data may suggest that some bacterial strains are radiosensitive and were eliminated by the TBI; however, the confounding variable exists that the response of the animal to TBI may have produced by cytokines or altered liver/biliary and pancreatic enzymes that disadvantage one or more of these strains of bacteria. Alteration of the immune system by TBI, production of inflammatory cytokines in the intestine that are deleterious to several bacterial strains, or facilitate overgrowth of other strains are possibilities. Studies with bacteria, yeast, and other microorganisms, when carried out in tissue culture, also must consider the possibility that the culture medium and the availability of nutrients may determine that one particular strain is advantaged, but not necessarily radiosensitive compared to another radioresistant strain. There have been clear demonstrations of multiple bacterial and yeast genes responsible for ionizing irradiation sensitivity, and clonal cell lines derived from these specific genetic variants, have led to the identification of a series of genes involved in the radiation response including prominent RAD50 and RAD51, which have shown counterparts in human cells in culture (See the chapter on “Origins of Life on Earth”). The important role of radioresistance genes in late radiation effects including carcinogenesis and leukemogenesis have also been established based on these studies initially performed in yeast.

Insects are generally radiation resistant; however, there is great variation within these categories as well. Genetics studies in Drosophila melanogaster (fruit flies) define the role of specific genes in the radiosensitivity of fruit flies.

Invertebrate species show a range of radiosensitivities. Studies with marine mollusks (Crepitula Fornicita) (3) demonstrated radiation adaptation of larvae to low doses of ionizing irradiation before the steep dose response curve leading to death that was observed. These studies suggested a phenomenon of adaptation to total body irradiation was occurring. Such adaptation was also seen in response of these mollusks to chemical toxins in seawater. In both situations, low doses resulted in accommodation of the species and more rapid growth and reproduction, whereas higher doses induced the rapid dose response curve for lethality as seen in inbred mice. There have been elegant radiobiology experiments carried out with plant species as well as

invertebrates. The germination of grass seed planted in chemically defined soil shows a similar pattern of radiation dose response curve with respect to number of seeds germinating and speed of growth as that shown by mollusks larvae adaptation to irradiation: Namely low dose irradiation increases growth and higher doses produce the dose response curve of kill.

This present web-based textbook focuses on principles applied to vertebrates, specifically the importance of medical countermeasures and radiation dosimetry for humans. Genetically inbred mouse strains also demonstrate a radiation adaptation to low doses of total body irradiation, which may influence survival. In genetic models of radiation-induced leukemia, mice exposed to a low dose priming total body irradiation event, show a subsequent decrease in leukemogenesis, when given the leukemogenic (higher) radiation dose at a subsequent time. Furthermore, mice administered a low dose of TBI have been shown to upregulate antioxidant stores, elevation in Manganese Superoxide Dismutase (MnSOD), catalase, glutathione peroxidase, and other enzymes associated with the neutralization of ionizing irradiation-induced free radicals (22-23, 28-29). These induction events of low dose total body irradiation may have primed the response of cells in tissues in advance allowing a radiosensitive tissue or organ to increase its intrinsic radiation resistance prior to exposure to a higher radiation dose. Total body irradiation experiments in larger vertebrates (dogs, swine, mini-pigs, and others) were carried out during the 1980s, and these data also reported. Many of these studies were carried out as part of the "Cold War" experiments and also during testing of the first Fission bombs in the late 1940's and 1950's. General principles on radiation-induced lethality were established for these larger animal species; however, the outbred nature of these animals obviated precise analysis, similarly to that carried out in genetic inbred mouse strains. General principles were established for the TBI responses of several species. The size of animals and relative position in the phylogenetic tree of the origin of species does not necessarily correlate to total body irradiation sensitivity. Readers should consult textbooks, which describe these differences. For scientists proposing to study the effects of total body irradiation, several general comments may help guide choices for planned studies.

Investigations of the genetic and molecular mechanisms of total body irradiation should focus on genetically inbred strains of mice, which are widely available and studies more reproducible.

Bone marrow transplantation studies after total body irradiation can be carried out in mice, rats, and other small rodents for studies of genetics and cellular and tissue interactions.

Studies related to human total body irradiation exposure and the development and validation of radiation countermeasures should consider studies in larger species including dogs, non-human primates, and swine. Due to the expense of managing research facilities for non-human primates (Rhesus Macaque, and Cynomolgus monkeys) – Readers should consult the **chapter by Mark Cline** on large animal radiobiology experiments, but at the same time the concern for the translation of data obtained in large animals to human radiation countermeasures. There has been recent interest in two other animal model systems for total body irradiation: the mini-pig (miniature swine) and the marmoset.

Studies of total body irradiation in both dogs, and non-human primates have established the principles of dose response curve for the hematopoietic syndrome. The $LD_{50/30}$ for outbred dogs

has been shown to be around 4.5 Gy, while that for Rhesus Macaque monkeys in the range of 9.0 Gy. Since human data on the $LD_{50/30}$, are more widely available from clinical bone marrow transplantation studies, and the data correlated with the available information from the Atomic bomb survivors (Hiroshima and Nagasaki in 1945), as well as radiation accidents, the $LD_{50/30}$ for humans has been established at around 4.5 Gy. This number as with any numbers for other experimental models described above is very much modified by and dependent upon radiation beam quality, dose homogeneity, as well as the age and gender of the exposed population.

Response of Humans to Total Body Irradiation

Much information has been gained from studies of clinical bone marrow transplantation patients. Total body irradiation was used initially in a single fraction delivered at low dose rate or fractionated (two treatments per day over several days). Radiation was delivered to "clear space" in the recipient bone marrow. This meaning has been taken literally in that the niche for engraftment of hematopoietic stem cells within the bone marrow consists of the stromal cell microenvironment, which is comprised of osteoblasts, other sets of bone marrow stromal cells, endothelial cells, neurons, bone marrow macrophages, and, of course, the bone spicules within the marrow. Total body irradiation, even if delivered at a low dose rate 3 – 4 cGy per minute, is (5-7) significantly lower than that used for clinical radiotherapy of tumors in specific regional (small volume) sites (200 cGy per minute). TBI has been delivered either in two opposed $Cobalt^{60}$ beams with field size opened to include the entire patient or with one beam and rotating the patient 180 degrees mid-treatment. Patients would sit in a knee to chest position and the low dose rate delivery for administration of around 1000 cGy (10 Gy), which at the low dose rate was determined to be a lethal dose required to clean space for bone marrow transplantation. The low dose rate decreased gastrointestinal cell damage. Other bone marrow transplant centers used a single radiation beam from a linear accelerator 4 MeV or 10 MeV with the patient placed on a gurney or stretcher again in a knee-chest position to allow the collimator of the linear accelerator to be opened and the field size to include the whole body. Because of the use of a single beam and dose and homogeneity, the patient would be rotated 180 degrees, so the entrance of the same radiation beam would deliver the dose half from each side.

The use of low dose rate irradiation was discovered in the 1970s to be critical for tolerance of the GI tract of patients. The bone marrow stem cells and other committed progenitors in the marrow (required to remove to "clear the space") were effectively removed by total body irradiation at 3 – 4 cGy per minute. However, gastrointestinal stem cells, the critical other population, which would not be replaced by bone marrow transplantation, tolerated the low dose rate radiation much better. Many publications on this topic are available. It should be noted that in more recent years, total body irradiation has been replaced (at many clinical marrow transplant centers) by using chemotherapy combinations of: Busulfan, Flutarabine, and L-Phenylalanine Mustard (Cytoxan), or other systemic chemotherapy drugs. Over the past 40 years, there has been a return to a use of total body irradiation, then back to chemotherapy, and this cycle has been repeated largely due to the concern in cancer patients for balancing the toxicity of total body irradiation, with the incomplete penetration of sanctuary tumor sites including those in the brain and spinal cord by chemotherapy drugs.

Clinical investigators in the 1970s reported the effects of total body irradiation on humans. These can be described as those subjective/symptomatic and objective based on the criteria of sampled tissues.

Clinical total body irradiation at the required low dose rate required patients to be immobilized for periods in excess of an hour to deliver 10 Gy (1000 cGy). There was often associated nausea and vomiting, diarrhea, flushing (redness of the skin), and discomfort of patients in the immobilized position for the radiation. Subjective quantifiable criteria included those parameters easily measurable in the peripheral blood. There was a significant increase in inflammatory cytokines reproducing data obtained in mouse and dog models. Rapid increases in hematopoietic cytokines including: G-CSF were rapidly detected once immunoassays were available for this hematopoietic growth factor. IL-1, TNF-α, TGF-β increased rapidly, as did other inflammatory cytokines. Routine measurements have been facilitated in recent years by use of the Luminex assay (Readers should go to the radiobiology methods section of this textbook to learn these techniques.).

Bone marrow transplantation by intravenous administration of bone marrow stem cells or subpopulations is usually carried out in clinical settings at around 24 hrs after total body irradiation. This interval is usually required to allow "clearing of space" induced apoptosis and elimination of hematopoietic cells in the bone marrow to allow sites for engraftment. A major discovery in recent years, has been the observation that cytotoxic therapy – for preparation of the host for marrow transplant can also be achieved by chemotherapy, which can "clear space" for injection of donor marrow cells.

In other marrow transplant recipients, with congenital defects in hematopoiesis, but no cancer, this is intrinsic deficiency in their own hematopoietic stem cells. Therefore, replacement with donor marrow can be achieved with lower doses of total body irradiation (in some cases, partial body irradiation) or lower doses of cytotoxic therapy. Such treatment protocols have been used in TGF-β sensitive Fanconi Anemia patients prior to marrow transplant.

In the case of a nuclear terrorist event from a fission bomb, the dose rate will be much higher, depending on the distance from the hypocenter and may exceed several Gy/second. Thus, the lethality from any given dose will be much greater.

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