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MEETING REPORT

Medical Countermeasures for Radiation Combined Injury: Radiation with Burn, Blast, Trauma and/or Sepsis. Report of an NIAID Workshop, March 26–27, 2007

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Non-clinical human radiation exposure events such as the Hiroshima and Nagasaki bombings or the Chernobyl accident are often coupled with other forms of injury, such as wounds, burns, blunt trauma, and infection. Radiation combined injury would also be expected after a radiological or nuclear attack. Few animal models of radiation combined injury exist, and mechanisms underlying the high mortality associated with complex radiation injuries are poorly understood. Medical countermeasures are currently available for management of the non-radiation components of radiation combined injury, but it is not known whether treatments for other insults will be effective when the injury is combined with radiation exposure. Further research is needed to elucidate mechanisms behind the synergistic lethality of radiation combined injury and to identify targets for medical countermeasures. To address these issues, the National Institute of Allergy and Infectious Diseases convened a workshop to make recommendations on the development of animal models of radiation combined injury, possible mechanisms of radiation combined injury, and future directions for countermeasure research, including target identification and end points to evaluate treatment efficacy. © 2008 by Radiation Research Society

INTRODUCTION

The White House Office of Science and Technology Policy's Radiological/Nuclear Threat Countermeasures Working Group rates radiation combined injury as a high-priority research area (*1*). A significant percentage of people exposed to radiation from radiological or nuclear terrorism are expected to sustain other injuries, including wounds, blunt trauma from blast overpressure, and burns, all of which may be complicated by microbial infections. Radiation exposure in animal models often worsens the development and progression of other injuries (*2*). To build on existing studies of countermeasures for other injuries, the Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, held a workshop on March 26–27, 2007 to address medical countermeasures for radiation combined injury. Investigators in the areas of radiation, burn, blast, trauma and sepsis were convened to identify research gaps and promote collaborations to understand mechanisms, discover targets, and develop medical countermeasures for radiation combined injury (Table 1).

Meeting presentations on the first day focused on potential scenarios for radiation combined injury, mechanistic studies, basic animal models, and overviews of historical and current research in the treatment of sepsis, burns, traumas and radiation combined injury. On day two, participants explored therapeutic agents for burns, wounds, infection and scarring and defined potential targets for drug development. After the presentations, an open discussion addressed animal models, assays and study end points for radiation combined injury that would move countermeasures toward Food and Drug Administration (FDA) licensure. Complementing several excellent overviews of radiation combined injury (*3–5*), this report reviews meeting outcomes to provide guidance on animal model development, possible physiological mechanisms of mortality of radiation combined injury, and the challenges of developing and licensing mitigators and treatments. Meeting slides can be viewed at http://www3.niaid.nih.gov/research/topics/radnuc/Meeting_Slides.htm.

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TABLE 1
Invited Workshop Speakers and Areas of Expertise[a]

Name	Affiliation	Expertise
Darrell Carney, Ph.D.	University of Texas Medical Branch	Thrombin peptides, wound healing
Carl Curling, Sc.D.	Institute for Defense Analyses	Nuclear scenario modeling, threat analysis
Harald Dorr, M.D.	Bundeswehr Institute of Radiobiology, Munich, Germany	Radiation accident medical management, late effects
Mark Ferguson, Ph.D.	University of Manchester	Skin injury, mechanisms of scar-free healing
John Fike, Ph.D.	University of California, San Francisco	Traumatic brain injury, radiation exposure, cognitive impairment
Martin Hauer-Jensen, M.D., Ph.D.	University of Arkansas for Medical Sciences	Radiation mechanisms and countermeasures for gastrointestinal injury
Erwin Hirsch, M.D.	Boston University School of Medicine	Trauma surgery, patient care
G. David Ledney, Ph.D.	Armed Forces Radiobiology Research Institute	Mechanisms of radiation combined injury, countermeasures
Paul Okunieff, M.D.	University of Rochester Medical Center	Radiation oncology, radiation skin damage, inflammatory responses
Terry C. Pellmar, Ph.D.	Armed Forces Radiobiology Research Institute	Radiation biology, countermeasure development, military response
Martin G. Schwacha, Ph.D.	University of Alabama at Birmingham	γδ T cells, burn injury, inflammatory responses
Alla Shapiro, M.D., Ph.D.	Office of Counter-Terrorism and Emergency Coordination, Food and Drug Administration	Clinical effects of radiation exposure, Chernobyl, FDA licensure pathways
Daniela Stricklin, Ph.D.	Swedish Defense Research Agency	Radiation gene expression, proteomics
Yongping Su, M.D., Ph.D.	Institute of Combined Radiation Injury, Chongqing, China	Radiation combined injury mechanisms, countermeasures
Peter Ward, M.D.	University of Michigan Health Systems	Microbiology, mechanisms of sepsis
Zhongmin Zou, M.D., Ph.D.	Institute of Combined Radiation Injury, Chongqing, China	Radiation combined injury mechanisms, countermeasures

[a] Invited speakers were given the opportunity to comment on the meeting report before its submission.

BACKGROUND

Human Radiation Exposures

A large number of events, including intentional bombings (Hiroshima and Nagasaki) and radiation accidents (e.g. Chernobyl and Goiania), have shown the importance of improving the diagnosis and management of radiation combined injuries. After the bombings of Hiroshima and Nagasaki, 60% to 70% of victims had thermal burns concurrent with radiation exposure (*6, 7*), and after the Goiania contamination (*8*) and the 1986 Chernobyl accident, the cutaneous component (e.g. radiation skin burns) of acute radiation sickness complicated clinical prognoses. For example, 115 Chernobyl victims developed acute radiation sickness, with 49% of these patients manifesting radiation burns (*9*). Skin involvement ranged from 1% to 50% of total body surface (Table 2) (*10*).

TABLE 2
Varying Severity of Skin Damage in Chernobyl Patients with Acute Radiation Syndrome

Severity (grade)	No. of patients	Percentage skin involvement in patients			
		50	11–49	1–10	Total
IV	20	9	10	1	20
III	21	3	15	3	21
II	43	1	9	2	12
I	31	0	1	2	3
Total	115	13	35	8	56

Note. Table provided by A. Shapiro, FDA, Silver Spring, MD. Originally published in UNSCEAR 1988 Report (*10*).

Of the 27 patients dying within 3 months of the Chernobyl accident, 19 had β-particle radiation burns over at least 40% of the body surface, and 22 died during a period of profound leukopenia 14 to 34 days after exposure (A. Shapiro). In 20 of these 22 patients, burns were the main cause of death; patients with more extensive β-particle radiation burns developed neutropenia earlier than other patients. Individuals with erythema and more than 40% body surface burns developed high fever and other symptoms of toxemia and hepatic and renal failure. The role of skin injury in mortality resulting from acute radiation sickness was also discussed (H. Dörr) with regard to analysis of the SEARCH databank (System for Evaluation and Archiving of Radiation Accidents based on Case Histories) (*11*). This analysis focused on the time course and severity of radiation-induced skin reactions and the extent of the skin surface affected. Consistent with clinical findings after the Chernobyl accident, the percentage of skin surface affected and the grade of hematological injury each correlated well with the clinical course of acute radiation sickness and were independent predictors of mortality.

Modeling Radiation Combined Injury

In developing effective countermeasures for radiation combined injury, it is imperative to understand the exposure scenarios anticipated in the wake of a radiological or

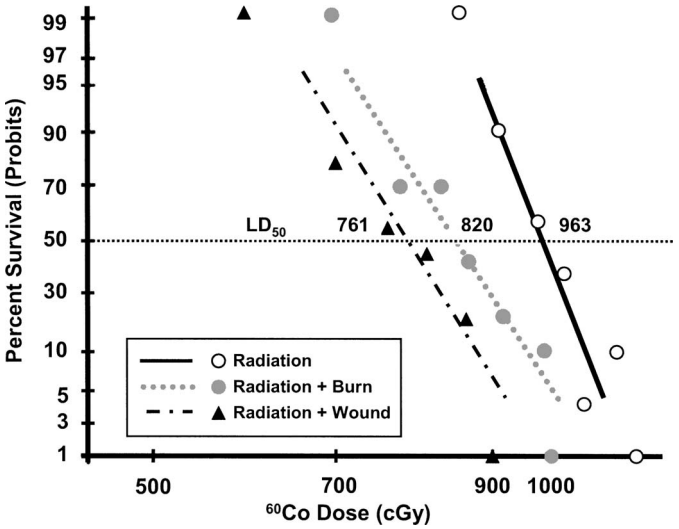

FIG. 1. The $LD_{50/30}$ for γ radiation is decreased by burn or skin-wound trauma in the mouse. Figure provided by G. D. Ledney, AFRRI, Bethesda, MD, as published in ref. (*18*).

nuclear event. Nuclear detonation energy is partitioned into ionizing radiation, thermal radiation, and blast pressure, which may cause wounding and other trauma (C. Curling). Different physical principles determine amount of energy transmitted in each form; the relative percentages of total energy absorbed by an individual from these three components are dependent on the distance from the point of detonation. An individual's location and orientation to the blast and shielding provided by buildings or other objects affect the degree and type of injury sustained, making it difficult to model human exposure precisely and to estimate numbers of casualties.

TRAUMA (BURNS OR WOUNDS) + RADIATION

Animal Studies

In animal models of radiation combined injury such as the rat (*12, 13*), guinea pig (*14*), dog (*15*), and swine (*16*), burns or wounds usually increase mortality after non-lethal radiation exposures. For example, rats exposed to 1, 2 or 5 Gy of X rays show significantly higher mortality when radiation is combined with an LD_{50} skin burn (*12*). Data demonstrating delayed wound closure after total-body irradiation of mice were presented (G. D. Ledney) (*17*). Wounds or burns also shifted the radiation survival curves for mice to the left, with resulting dose modification factors (DMFs) of 1.3 and 1.2, respectively (Fig. 1) (*18*). Animals exposed to radiation with wounding are more susceptible to infection and experience delayed skin healing and decreased survival (*19*). Because radiation impairs immunity and repair, addition of cutaneous injury increases the risk of infection, enhancing morbidity and mortality compared to radiation alone (*20*).

Other experiments confirmed these findings, showing that the average wound healing time increases with radiation doses greater than 4 Gy compared to unirradiated wounds (*21*). Paradoxically, wounding of mice 24 h prior to irradiation improved 30-day survival compared to unwounded animals (*17*), a finding attributed to an increase in clonogenic myeloid elements. Wounding after irradiation decreased survival, consistent with the previous study. For this reason, it is imperative to evaluate wounding both prior to and after radiation exposure in selected animal models to determine whether similar mechanisms exist.

Ongoing animal research also includes the interaction of radiation exposure with brain injury. Radiation-induced cognitive impairment is thought to involve hippocampal neurogenesis (*22*). For example, low doses of X rays reduce new neuron production in a dose-dependent fashion (*23*). Recent data suggest that when neurogenic microenvironments are ''primed'' by pre-existing oxidative stress, the deleterious effects of radiation on neurogenesis are blunted (*24*). This effect may involve alterations in inflammatory cell function, rendering them protective rather than damaging. In preliminary studies, irradiation of mice induced a modest reduction in hippocampal neurogenesis, while traumatic brain injury alone had a larger impact. Surprisingly, when focal brain injury occurred 1 month after radiation exposure, more newly born neurons survived than after brain injury only (J. Fike, unpublished data), suggesting that prior irradiation protected neurogenic cells. Understanding the mechanisms of this protection may lead to the development of strategies to ameliorate the cognitive consequences of exposure to radiation and/or traumatic brain injury.

Skin Wounds and Radiation

Normal wound healing is categorized into four processes: hemostasis, inflammation, proliferation and remodeling. Chronic wounds or pathological scarring may develop if these events are impaired by confounding injuries such as radiation exposure or infection. The pathological mechanisms of impaired and delayed wound healing after irradiation are complex and depend on the radiation dose, energy and type (e.g., photons or neutrons) as well as on the extent of the body surface affected. Total-body irradiation followed by wounding reduces acute inflammatory responses, as manifested by decreased inflammatory cells and impaired cellular function compared with wounding alone (*5*). Radiation also inhibits the proliferation phase by reducing the number and function of fibroblasts and vascular endothelial cells, delaying re-epithelialization, and affecting remodeling. Thus, in the acute phase, radiation exposure can prevent healing, resulting in chronic wounds (*25*). Late radiation skin effects such as fibrosis are attributable in part to endothelial cell death and to loss of dermal vasculature.

Mechanisms

Studies on molecular mechanisms of skin radiation damage suggest a role for cytokine feedback (P. Okunieff). Cy-

tokine homeostasis and cross-talk are controlled in a complex manner, and imbalances in feedback mechanisms after radiation exposure can occur. In fact, radiation exposures alter levels of multiple cytokines compared to unexposed controls (D. Stricklin, unpublished data). To further complicate matters, cytokines may be deleterious or beneficial depending on the context (tissue cytokine concentration, phase of wound healing, etc.). Radiation-induced increases in IL1 and TGFβ1 expression are predictive of fibrovascular changes after high doses of radiation (*26*), as shown in several mouse strains (*27*) and in human patients (*28*). Different strains of mice or knockout mice show differing radiation sensitivities based on their TGFβ levels. IL6 is also enhanced in mice exposed to radiation and thermal burns (*29*). These data suggest that therapies that reduce IL1, IL6 and/or TGFβ expression might alter the late fibrovascular effects of radiation exposure, enhancing short-term survival. Consistent with these findings, COX2 inhibitors reduce IL1 and macrophage chemotaxis into tissues and minimize short- and long-term inflammatory effects in irradiated skin (*30*). Pentoxifylline (*31*), curcumin (*26*) and esculentoside A (*32*) also reduce levels of IL1α, TGFβ and other inflammatory cytokines, resulting in radiation protection of soft tissue.

Major thermal injury induces an immuno-pathogenic response, leading to delayed wound healing, increased susceptibility to sepsis, and multi-organ failure (M. Schwacha) (*33*). Although many mediators and cells regulate inflammatory processes after injury, the balance between inflammatory and anti-inflammatory responses is disrupted after burning (*34*). Recent studies implicate γδ T cells in the induction of organ injury after burning (*35*). These cells are part of the innate immune system and are important in early inflammatory/immune responses. In a mouse model of thermal injury, cytokine induction by γδ T cells is important in neutrophil-mediated damage in the lung and gastrointestinal (GI) tract (*36*). Conversely, γδ T cells are also important in wound healing processes through immune surveillance and tissue repair. Severe injuries such as burns initiate an exaggerated inflammatory response and induce multi-organ failure, particularly if other inflammatory stimuli are encountered. Based on burn severity, additional injuries may exacerbate immunological complications.

Multi-organ Injury, Endothelial Dysfunction and Radiation Combined Injury

Recent attention has been focused on the radiation-induced multi-organ dysfunction syndrome. Diffuse vascular injury and endothelial dysfunction may be important connecting factors in this complex condition. High doses of radiation cause an immediate increase in capillary permeability (*37*). Endothelial dysfunction is important in the pathophysiology of many aspects of radiation combined injury, including radiation, burns, shock, blunt and penetrating trauma, multiple organ dysfunction, and sepsis (*38*) (M. Hauer-Jensen). Endothelial dysfunction is also implicated in radiation damage to the GI tract and is responsible for depletion of epithelial cells, breakdown of the mucosal barrier, and decreased crypt cell proliferation (Fig. 2) (*39*). In early work on the time of death from radiation and skin burn, GI injury, not hematopoietic syndrome, led to mortality, even though combined injuries did not affect the mucosal mass or crypt cell numbers beyond the damage observed for radiation alone (*40*). Paris *et al.* suggested that radiation-induced apoptosis of endothelial cells may be the primary cause of GI damage (*41*); however, other results contradict this finding (*42*).

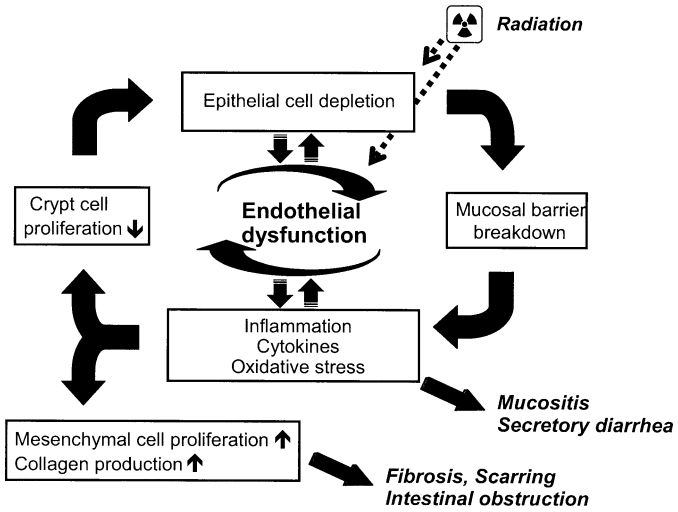

FIG. 2. Model showing how interactions between epithelial and endothelial radiation injury in the intestine causes endothelial dysfunction, exacerbates acute intestinal radiation toxicity, and subsequently sustains the cycle of chronicity of intestinal radiation fibrosis. Figure provided by M. Hauer-Jensen, from ref. (*39*) with permission from Nova Science Publishers, Inc.

General Countermeasures for Radiation Combined Injury

Prior administration of WR-151327, an aminothioate known to be an effective radioprotector, increased survival in mouse models of radiation combined injury (*43*). WR-151327 protected animals given radiation alone (DMF = 1.53) as well as animals subjected to radiation combined injury (DMF = 1.51). Other compounds such as pentoxifylline, glycine and gadolinium chloride had no effect on survival of mice and rats given 7 Gy radiation plus a full-thickness (10% in mice or 15% in rats) total-body surface burn (*44*). In contrast, anti-IL6 antibodies in the same mouse model enhanced survival by up to 60% over that of nontreated controls (*29*).

1. Treatments for skin damage from radiation combined injury

After skin injury, major medical challenges include the acceleration of healing and minimization of scarring. Treatments evaluated for radiation skin damage include linoleic

acid, topical/systemic steroids, systemic pentoxifylline and α-tocopherol, hydrocolloid dressings, and thrombocytic growth factors (*45*). In the absence of other countermeasures, these compounds, in conjunction with supportive care, are expected to be the first therapies used; however, several new compounds in preclinical and clinical development for treatment of non-radiation skin injuries might also be appropriate for treating radiation combined injury. For example, estradiol treatment markedly accelerates healing of punch biopsy wounds in animal models and humans (*46*), and studies in mouse and rat embryos have identified cellular and molecular mechanisms that differ in embryonic (scar-free) and adult (scar-forming) healing (*47*). This research has led to the identification of novel therapeutics such as human recombinant $TGF\beta_3$, which when administered by intradermal injection at the time of injury, prevents scarring (now in clinical trials, according to M. Ferguson). Two other drugs in clinical trials for scar reduction include a small molecule antagonist of $TGF\beta_1$ and β_2 and a formulation of human recombinant IL10, which acts as an inflammatory modulator.

Another potential treatment for radiation-induced skin damage is TP508, a non-proteolytic peptide from the human thrombin receptor binding domain (*48*). In addition to its effects on coagulation, thrombin increases vascular permeability and stimulates inflammatory processes (*49*). TP508 accelerates repair and revascularization of wounds in irradiated rats, suggesting effects on circulating progenitor and inflammatory cell recruitment (*50*). In pilot clinical trials in diabetic ulcers, TP508 improved wound closure and nearly doubled healing rates relative to placebo treatments (*51*). This compound also induces angiogenesis and enlists other reparative factors, an action important for wound healing, since endothelial dysfunction associated with chronic wounds often limits the angiogenic and proliferative effects of growth factors. TP508 activity affects nitric oxide signaling pathways, reducing endothelial dysfunction (D. Carney). Reversal of endothelial dysfunction is a common thread among the seemingly diverse beneficial effects noted for TP508 treatment, including enhanced bone regeneration (*52*) and amelioration of heart damage after ischemia (*53*).

2. *Treatment of GI dysfunction from radiation combined injury*

Radiation also causes endothelial dysfunction by reducing expression of endothelial thrombomodulin, a transmembrane protein receptor that regulates inflammation and is found on endothelial cells (*54*). Statins increase levels of endothelial thrombomodulin (*55*), thus reducing radiation effects and decreasing GI injury (*56*). Statin use is also linked to reduced mortality after major operations (*57*), minimization of postoperative inflammation (*58*), reduction of E-selectin levels in patients with severe burns (*59*), and decreased mortality in sepsis (*60*). Other agents to prevent and treat GI injury were discussed, including somatostatin analogs (*56*) and glucagon-like peptide-2 (*61*), which enhance crypt cell proliferation and reduce GI infection after radiation exposure.

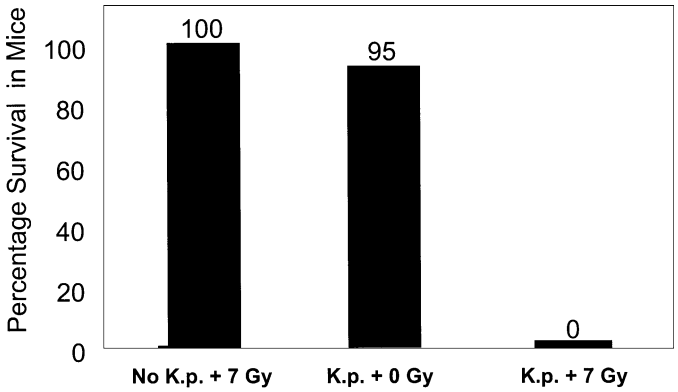

FIG. 3. Radiation exposure decreases the survival of mice injected with 1.1×10^5 *Klebsiella pneumoniae* (K.p.) cells. Figure provided by T. Pellmar, AFRRI, Bethesda, MD (T. B. Elliott, unpublished data).

INFECTION + RADIATION

Bacterial sepsis in humans is a major cause of morbidity and mortality, affecting about 600,000 patients in the United States each year (P. Ward). Aside from standard supportive therapies such as ventilators and vasopressors, the only currently approved specific therapy for sepsis is activated protein C (*62*). The microorganisms chiefly responsible for sepsis in humans are gram-positive and gram-negative bacteria and fungi (*63*). Radiation-exposed individuals are more susceptible to these pathogens, fewer microbes are needed to establish an infection, and clinical manifestations are more severe. In addition, even weeks and months after radiation exposure, latent infections are more prevalent in radiation-exposed animals than in unirradiated controls (*64*).

Ionizing radiation substantially increases sepsis risk by suppressing the hematopoietic system, leading to decreased survival. As shown in Fig. 3, mice irradiated concurrent with *Klebsiella pneumoniae* injection exhibited 0% survival, while 100% of mice exposed to radiation alone and 95% with infection alone survived. Dr. Peter Ward discussed how abdominal irradiation of mice reduces normal flora while greatly increasing the numbers of Enterobacteriaceae associated with lethal sepsis (*65*). This increased risk of sepsis likely reflects a combination of apoptosis of lymphoid tissues and the resulting immunosuppression, a systemic inflammatory response syndrome with high levels of pro-inflammatory mediators, complement-induced multiorgan dysfunction, and loss of innate neutrophil functions. Streptomycin (*16, 66*), ceftriaxone (*67*), ofloxacin and oxacillin (*68*) treatments in animal models of radiation combined injury increase survival after radiation combined injury, as do silvadene (*69*) and gentamicin antibiotic creams

alone or in combination with the immunomodulator synthetic trehalose dimycolate (*43, 68*). Some testing in models of radiation combined injury has been done with current anti-bacterial agents such as quinolones (*70*), and ciprofloxacin and levofloxacin are available from the strategic national stockpile for treatments in a mass casualty setting. However, more work must be done, and new anti-viral and anti-fungal agents should also be explored.

As demonstrated by the data presented above, immunomodulatory and pro-inflammatory effects are important in the effects of radiation combined injury. Activation of similar systemic responses to different types of injury may lead to an amplification of host response, producing the synergistic lethality associated with radiation combined injury. For example, trauma activates changes in pro- and anti-inflammatory cytokine concentrations similar to those triggered by sepsis, suggesting a universal response to systemic inflammatory conditions (*71*).

CONSENSUS DISCUSSION

After completion of formal presentations, panelists and meeting participants discussed the selection of animal models for radiation combined injury and the induction of radiation combined injuries. Their recommendations are outlined below.

Selection of Animal Models

Participants agreed that the first species for testing a countermeasure should be a genetically defined small animal, preferably the mouse. Mouse strains discussed during the workshop included B6D2F1 and C57BL6, with the latter strain emphasized due to its well-established genetic background and the availability of knockout and transgenic animals of this genetic background. The parental strains of hybrid B6D2F1 mice are the genetically defined DBA/2 mouse and the C57BL/6 mouse. Animals should be maintained in a clean facility and should be free of disease; however, microbe-free environments might not be realistic in the wake of a nuclear or radiological incident. Pre-existing disease states in animals, such as herpes, cytomegalovirus infection, diabetes and autoimmunities, need to be explored because similar syndromes would be expected in exposed human populations. The panel emphasized that several animal models would be needed, with some models lending themselves more readily to particular studies and outcomes.

With regard to large animal models, participants felt that swine are the most appropriate, given the similarity of their skin to human skin. Although there are swine data available from field studies (e.g., nuclear detonations carried out by the military) and very early laboratory work (*72–74*), there is little current research on the effects of radiation alone in this animal model. These studies would require updating, taking into account new supportive care measures, before appropriate swine models, particularly miniature swine strains, could be validated.

In establishing animal models of radiation combined injury, researchers should start with established models for the confounding injury (e.g. burn, blast, wound, trauma or sepsis), observing how that specific injury is modified by radiation. Radiation plus chemical exposure is another possible scenario of radiation combined injury for which prior animal research is available (*75–78*). Participants also discussed potential research complications from pre-existing genetic conditions in certain animal models, for example, endothelial dysfunction in Yucatan hyper-cholesterol pigs.

Supportive Care

Considerable discussion focused on the importance of supportive care in animal models of radiation combined injury. Basic supportive care, even provided in a limited fashion, after a mass casualty event would be an effective first approach to treating radiation injuries (T. MacVittie). Many aspects of basic support may not need to be administered immediately (e.g., antibiotics and blood products can be administered when indicated clinically). Although intensive care is unlikely to be feasible for mass casualty care after a radiological or nuclear disaster, basic supportive care is generally considered achievable. However, the development of radiation countermeasures should take into consideration the fact that supportive care may be unavailable; a drug that is effective without supportive care would provide additional flexibility in a real event. Rodent studies provide the opportunity to evaluate the efficacy of countermeasures without additional support. Efficacy without support can then be compared to supportive care alone or the drug with supportive care. In non-human primate or canine models, supportive care (e.g., intravenous fluids, blood products, anti-emetics and analgesics) may be required, precluding these types of analyses.

Consideration of Exposed Populations

Few data for acute high-dose radiation exposures in pregnant women, children and the elderly exist, and even less information is available about radiation combined injury in these populations. Although some animal models for children exist, these populations present difficult treatment challenges because they respond differently to radiation than healthy adults (*79*). Gender and racial background also influence responses to radiation and other co-morbidities. For example, clinically, females experience increased mortality after thermal injury (*80*); however, female rodents (*81*) and humans (*82, 83*) are resistant to the development of sepsis, possibly due to the engagement of estrogen receptors. In addition, the incidence of post-wound keloids varies by race, with a 15-fold greater risk of keloid formation in darker-skinned compared to lighter-skinned individuals (*84*). The impact of treatments on different pop-

ulations therefore requires investigations in new animal models.

Modeling Aspects of Radiation Combined Injuries in Animals

With regard to creating different radiation combined injuries, meeting participants suggested the following guidelines.

1. Radiation exposure

Radiation dose–response curves should be established for the selected animal model with appropriate supportive care. Researchers should then select a radiation dose that yields 70 to 80% mortality (LD_{70}–LD_{80}) to study countermeasure efficacy. Radiation quality and dose rate should also be considered, with exposures selected that would be expected after a radiological or nuclear event. Prompt radiation may include γ rays or mixed neutrons-γ rays, while fallout exposures could also include α and β particles.

2. Infection

Klebsiella and *Pseudomonas* given as an airway challenge in mice should be used, since the doses, clearance and lethalities of these agents are well established. Although *Klebsiella* and *Pseudomonas* represent well-characterized gram-negative strains, they might not represent infections anticipated in the wake of a radiation event, specifically gram-positive *Staphylococcus* and *Streptococcus* bacteria. For this reason, human databases for radiation exposure such as SEARCH (*11*) should be explored to determine what infections would be expected.

3. Wounds

In separate experiments, incisions should be made before and after radiation exposure to determine if effects are the same, and the injury site should be colonized with defined flora/bacteria to promote an infection, allowing standardization of treatment. However, researchers must be aware that clean wounds might not affect radiation responses in the same way as infected wounds. Treatment of acute wounds in small and large animals show good correlation with treatment of wounds in human patients; however, models of chronic treatment of wound injuries (e.g., diabetic or venous ulcers and pressure sores) are less well established, and these models may require additional validation.

4. Burns

Wet (hot water scalding) or dry (branding) methods are appropriate to create burns. Flash burns inflicted by an arc lamp to simulate the flash from a nuclear weapon represent another option for creating burn injury. Clinically, partial- and full-thickness burns would be expected after a nuclear or radiological event; however, developing a partial-thickness burn model acceptable under current Public Health Service guidelines for the humane care and use of animals (*85*) represents a challenge. In addition, because radiation burns and thermal burns are different (thermal burns occur in minutes, whereas radiation burns evolve over days to weeks), both types of burns should be considered but should be modeled separately.

Development of Countermeasures for Radiation Combined Injury

In the absence of ethical human efficacy studies, countermeasure studies must be done in animals with responses similar to those of humans. Drugs should first be assessed independently for each confounding injury (e.g. radiation, burn, wound or infection alone) and then tested for radiation combined injury in the same models. For FDA approval under the ''animal rule'' (*86*), any treatment effects noted in a rodent must be linked to a larger animal, and responses in both models must be further correlated to an anticipated human response. Under certain circumstances, however, a single non-rodent animal model might be sufficient for approval. Although data may be available for the use of countermeasures in humans, FDA animal rule pathways may still be required, because the localized, high-dose, fractionated radiation given to humans during therapeutic irradiation and the subsequent responses of the patients to these treatments might not be predictive of what would be expected in a terrorist event involving a healthy population. In addition, moderate countermeasure toxicities may not be acceptable in individuals who receive drugs in error.

The FDA will likely require that countermeasure studies demonstrate enhanced survival or increased mean survival times as a primary end points. Secondary end points of interest might include hematopoietic parameters, changes in cytokine profiles (which may provide data about mechanisms), time of wound healing, bacterial clearance, and physical signs and symptoms such as severity of diarrhea and vomiting. Other relevant, injury-specific end points might include time to wound closure, healing time of burns, and bacterial clearance.

In summary, existing injury models and products to treat burns, wounds, infections and trauma should be the foundation for developing animal models of and countermeasures for radiation combined injury. Optimum treatments for radiation combined injury may involve broad-spectrum anti-microbials and agents that interact with pathways that are shared between different forms of injury (e.g., those that target endothelial dysfunction). No single countermeasure will likely suffice; instead, a cocktail of different compounds may be necessary to address the complications of radiation combined injury, including sepsis, with different drug combinations possibly required for different forms of radiation combined injury. Eventual FDA licensure of any product for this indication will depend on understanding

the pathways and synergy involved in generating radiation combined injury and defining the mechanisms by which a proposed countermeasure enhances survival.

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