RADIOBIOLOGY RESEARCH:

AN EXPERT PANEL REVIEW CONDUCTED BY

THE NATIONAL INSTITUTE OF ALLERGY

AND INFECTIOUS DISEASES

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EXECUTIVE SUMMARY

There are increased concerns regarding the potential of terrorists using biological, chemical, or radiological agents against the civilian population. In the past the Department of Defense maintained a research and development program which addressed these threats for military forces, including primary responsibility for countermeasures related to radiological threats. The National Institutes of Health (NIH) is actively assessing relevant opportunities to exploit medical breakthroughs and focus its efforts on the development of new and effective countermeasures for all subsets of the U.S. population. On behalf of the National Institutes of Health (NIH), the National Institute of Allergy and Infectious Diseases (NIAID) convened a special meeting of experts on February 26, 2003, in Bethesda, Maryland, to review ongoing research efforts in development of medical countermeasures to radiological threats.

The purpose of the meeting was to: (1) provide NIAID with an overview of current radiobiology research; (2) identify gaps in research that are critical to the development of specific medical products to protect the civilian population from a radiological threat; and (3) explore ways in which the NIAID/NIH can collaborate in current radiological research efforts. With the assistance of organizations such as the National Cancer Institute, the Armed Forces Radiobiology Research Institute, and the Department of Energy, gaps were identified and short-term and mid-term research priorities were recommended. The results of this meeting will be used by the NIH Biodefense Research Coordinating Committee, as efforts across NIH are coordinated.

The participants recommended that emphasis be placed on:

- Biodosimetry
- Automated diagnostic systems
- Radioprotectant drugs and treatments
- Antibiotics for use following radiation exposure
- Research resource support, facilities, personnel, model systems

In addition, the participants recommended that priorities be established for the development of products that appear to have the greatest promise based on need, efficacy, and safety as part of a national strategy aimed at protecting all subgroups of the U.S. population.

BACKGROUND

In response to the growing terrorist threat to the United States of ionizing radiation, the National Research Council of the National Academy of Sciences initiated a review of the role of science and technology in December 2001. In its 2002 report entitled "Making the Nation Safer: The Role of Science and Technology in Countering Terrorism," the committee defined three plausible threat scenarios: (1) attacks involving the detonation of nuclear weapons or an improvised nuclear device; (2) attacks on nuclear reactors or spent nuclear fuel sources; and (3) attacks involving radiological dispersion devices (RDDs). The wide dispersal of radiation that could be generated by an RDD combined with explosives was of special concern, due to poorly monitored supplies of radionuclides and nuclear wastes outside the U.S. and the potential illegal shipment of such sources of radiation into the U.S. Recommendations were made on improved surveillance, detection, and control measures and increased medical capabilities to address such emergencies. However, the medical products that would be needed to minimize morbidity and mortality during and after a radiological incident were not addressed.

On February 26, 2003, the Office of Biodefense Research Affairs of NIAID's Division of Microbiology and Infectious Diseases, convened a group of highly respected scientists and radiological experts currently engaged in radiation-related research. The one-day meeting was held at the Uniformed Services University of the Health Sciences in Bethesda, Maryland. Participating in this meeting were representatives from the National Cancer Institute (NCI); the Armed Forces Radiobiology Research Institute (AFRRI); the Food and Drug Administration (FDA); the Department of Energy (DOE); the Office of Public Health Preparedness and Emergency Response in the Departmentof Health and Human Services (DHHS); and the Office of Homeland Security (The White House).

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Two recent scientific workshops provided an excellent background for the presentations and discussions at the meeting. NCI's Radiation Research Program sponsored a workshop in September 2000 entitled "Modifying Normal Tissue Damage Post-Irradiation," which focused on the larger doses of radiation encountered in radiation therapy. A second workshop entitled "Molecular and Cellular Biology of Moderate Dose Radiation and Potential Mechanisms of Radiation Protection" was convened in December 2001 to address progress in radiobiology, dosimetry, and therapeutic options to prevent or ameliorate radiation damage to normal tissues.

This report identifies and discusses specific knowledge gaps in the areas of biological effects of radiation, biodosimetry, radioprotectant drugs, and post-exposure therapeutics. Short- and mid-term research priorities are outlined to begin to address the gaps. Recommendations focus on the research and development of diagnostics, radioprotectants, and therapeutics for radiation exposures. Additionally, the report addresses research resources, models, and scientific manpower needed to conduct research and development efforts to address radiological threats.

BIOLOGICAL EFFECTS

Dose-related biological responses and mechanisms of injury from low-dose exposure (1 Gy or less; Gy = Gray, or unit of absorbed dose of radiation) to ionizing radiation are not as well characterized as those responses observed following moderate- to high-dose exposure. It is anticipated that many of the more obvious biological affects might be reflected in the hematopoietic system, such as mild granulocytopenia with doses as low as 1-2 Gy. However, less obvious biological changes may occur in the less radiosensitive tissues and organ systems, such as the gastrointestinal mucosa and the cardiovascular system. Longitudinal epidemiologic research to assess long-term health risks from low-dose radiation exposure among x-ray technicians, nuclear weapons test fallout subjects, and the general population in the former Soviet Union living in close proximity to nuclear power plants is in progress. Federal research programs focus on the molecular and cellular aspects of low-dose radiation research science so dose-response relationships and associated health risks can be better defined. Epidemiologic and laboratory data may provide the scientific basis for developing radiation protection standards for chronic low-level radiation exposure. The amount of radiation above the nominal background that is considered harmful is the subject of continued scientific investigation.

- **Studies should continue to quantitate the degree and type of damage that occurs following acute and sustained low-level exposure below 1 Gy.**

The molecular mechanisms of genetic damage to cells and tissues, and the limitations of cells to successfully initiate and complete DNA repair, are not well understood. The relative increased susceptibility of bone marrow cells to injury compared to that of mucosal cells of the gastrointestinal tract may reflect different levels of ability to successfully initiate repair. Since it is assumed that the immunological changes following radiation exposure are unlike those seen following exposure to an infectious agent, an understanding of innate mechanisms of protection and repair is of paramount importance. Efforts to identify and minimize "bystander" effects of low-dose radiation exposure at the molecular, cellular, and tissue levels are continuing in the clinical setting. The bystander effect refers to radiation-induced effects in adjacent or nearby unirradiated cells. These effects depend on intercellular communication, and imply an amplification of the consequences of the original event. Radiation-induced tissue effects are progressive over time, a finding supported by clinical experience; this suggests that the response to radiation and subsequent repair and/or healing is multi-factorial. Therefore, the often-unavoidable collateral bystander damage caused by radiation therapy to normal tissues provides an opportunity to investigate and understand susceptibility and the limits of the repair process. The effects of radiation on cells and tissues are now being investigated with sophisticated molecular biological tools and proteomic technology. Scientists should continue to investigate the variety of repair mechanisms that function at the cell and tissue level. These investigations should take into consideration the possibility of genetic transformation in tissues with increased susceptibility to radiation-induced damage.

- **Improved techniques are required to determine the type and degree of direct and bystander radiation effects occurring at the molecular,**

cellular, tissue and organ levels following exposure to ionizing radiation, as well as a more detailed assessment of the repair mechanisms.

Radiation exposure combined with an infectious or chemical agent may place individuals at increased risk of injury or death. The effects of high-dose radiation exposure have been characterized in atomic bomb survivors in Japan and in people acutely exposed following the 1986 Chernobyl nuclear reactor incident. The effects of radiation combined with other weapons of mass destruction (e.g., an RDD used with a biological or chemical agent) have not been studied. There may be synergistic effects, or delays in the healing process. Immunosuppression can clearly result from irradiation, but certain drugs and chemicals can also cause this. Delivery of a less than lethal dose of radiation may increase morbidity and mortality due to immunosuppression or could be combined with direct organ/tissue toxicity. A study conducted at AFRRI indicates that mice exposed to a 7-Gy dose of radiation experience higher mortality following intratracheal challenge with the Sterne strain of *Bacillus anthracis*. While it is generally accepted that moderate to high doses of radiation increase host susceptibility to infectious agents, there is little information on the combined or synergistic effects of radiation exposure and biological toxins or chemical agents.

- **More in-depth studies on the effects of the concomitant effects of sub-lethal doses of ionizing radiation with infectious and/or toxic agents need to be conducted to determine the pathways of injury, overall morbidity, and mechanisms of recovery.**

BIODOSIMETRY

Qualitative and quantitative assessments of low- and moderate-dose radiation exposure are needed to determine health effects. Biodosimetry provides biological information that is necessary for medical decision-making, beyond the important measure of physical exposure. Methods to quantitate moderate doses of radiation exposure ranging from 1 to 10 are more fully characterized than are those for low-dose exposures (<1 Gy). The current validated biological standard to evaluate low dose radiation exposure is the "lymphocyte dicentric assay," which quantitates the formation of dicentric chromosomal aberrations in metaphase spreads of mitogen-stimulated lymphocytes from individuals following irradiation. Dicentric chromosomal aberrations in peripheral blood lymphocytes are useful biomarkers for ionizing radiation exposure, since background levels in the general population are very low (1 per 1000 metaphase spreads). Furthermore, because radiation exposure *in vitro* and *in vivo* produces similar levels of dicentric chromosomal aberrations per Gy, an *in vitro*-generated calibration curve allows assessment of radiation dose to an exposed individual. Doses of approximately 2 Gy eliminate lymphocytes from the circulation. However, all of the factors that influence the minimum dose of radiation required to produce dicentric chromosome fragments are not completely understood.

Automated instrumentation to accomplish sample preparation and analysis of dicentric chromosomal aberrations is at the preclinical research phase of development. A simple, reproducible, and automated high–throughput differential blood cell counter for rapid evaluation

of dicentric chromosomal aberrations may be particularly useful for estimating dose following an unexpected radiation exposure in the general population. Sophisticated quantitative assays to identify and validate molecular biomarkers of radiation exposure using proteomic and genomic technologies are still in the early phases of research and development. Thus, it may be some time before a clear clinical correlation between ionizing radiation dose and putative biomarker expression is achieved.

- **New, sensitive assays to quantitate exposure to low and moderate doses of radiation and the degree of cellular and genetic damage are needed.**
- **Attention should be directed toward the development and validation of automated systems that allow rapid analysis of large numbers of specimens.**

RADIOPROTECTANT DRUGS

The employment of physical protective measures, such as suits and masks, may not be as useful in an unpredicted radiobiological emergency as in an industrial setting involving predictable exposure to known hazardous chemicals. Heavy lead shielding does provide physical protection against directed sources of ionizing radiation, but contamination following a terrorist release of radionuclides could result in a range of acute exposure doses as well as chronic exposure.

Energy transfer from ionizing radiation to cells and tissues results in the formation of free radicals and reactive oxygen species along the path of the radiation. These molecules can induce potentially dangerous secondary changes in cells and tissues. If radiation exposure is anticipated, a potential preventive strategy to limit the damage is pretreatment with "scavenger" drugs to eliminate the effects of free radicals and other reactive molecules. Aminothiols represent one of several families of compounds that have been shown to be “radioprotective” when administered prior to radiation exposure. One promising compound within this class is amifostine, which has been licensed for use in cancer radiation therapy and chemotherapy when administered inravenously. Use of drugs such as amifostine as a pretreatment for radiation exposure will require more research to document safety and efficacy. Drugs that must be administered intravenously may be acceptable in the clinical setting but are not as practical in the field or in a mass-casualty situation. Another promising compound is the radioprotective steroid 5-androstenediol. This androstene steroid is associated with low toxicity and long action and has been shown to enhance survival after exposure to 10.5 Gy of radiation in the mouse model. Nitroxides are also effective radioprotectants that are currently in clinical trials to evaluate their anti-carcinogenic potential. Other possible radioprotectants that require further evaluation include anti-oxidants, nutraceutical drugs (e.g., vitamin E analogs and soy isoflavone), and benzylsulfone analogs.

Potassium iodide (KI) is a licensed drug that functions by blocking radioactive iodine deposition in the thyroid. To be most effective, KI should be taken several hours before exposure or immediately after inhalation or ingestion of radioactive iodine. KI does not protect individuals from external radiation sources or from the effects of isotopes other than iodine.

- **Radioprotectants should be developed that are easily administered and broadly active, and have minimal side effects even with prolonged use.**

POST-EXPOSURE TREATMENT

The amount of radiation received is greater when radioisotopes are inhaled and/or ingested than when they are absorbed through the skin. Radiation exposure is associated with both acute and chronic effects, and treatment will vary with the specific radionuclide involved and the amount and route of exposure. Consideration must be given to the target tissue(s) at greatest risk; cells that reproduce most rapidly are the most sensitive to damage. Regardless of the route of exposure, therapeutic intervention is time-sensitive and dependent on early recognition of an incident.

Prussian Blue is an investigational drug that has been used since the 1960s to enhance the excretion of cesium isotopes. Oral administration of Prussian Blue prevents the intestinal absorption of cesium, which is subsequently excreted. Ion exchange resins such as sodium polystyrene sulfonate may have some usefulness, but they have not been approved by FDA. Aluminum hydroxide, which limits the uptake of strontium-90, must be given immediately after ingestion of this radioactive material because of its rapid absorption and incorporation into bone and tissues. Chelating agents such as ethylenediamineatetracetic acid (EDTA) and diethylethylenetriamine-pentaacetic acid (DTPA) bind quickly to isotopes of metals and form soluble complexes that are then excreted by the kidneys. DTPA is administered intravenously and has been used for chelation of plutonium, berkelium, californium, americium, and curium.

- **The identification of safe and effective therapeutics directed at radionuclides most likely to be used as terrorist weapons should be accelerated.**

After radiation damage of the hematopoietic system, growth factors and colony-stimulating factors that stimulate the production of lymphoid cells may aid in recovery and provide added protection against infection. Several pharmaceuticals are approved for use in patients who are either leukopenic or anemic as a consequence of cancer chemotherapy. Other promising compounds are in various stages of clinical evaluation.

- **Licensed drugs that are specifically intended to stimulate hematopoiesis should be evaluated for use in post-radiation injury.**

Radiation-induced damage of the gastrointestinal mucosa (destruction of crypt cells in the epithelial lining) can result in malabsorption, hemorrhage, and increased susceptibility to infection. Some patients can survive this type of injury despite the resulting excessive fluid loss and electrolyte imbalances, but their ability to control infections is severely compromised. Little data exist to define the antibiotics most useful in managing infections following radiation injury. In addition, new treatment options should be explored, including the use of probiotics.

- **Animal models of radiation injury and infection should be developed and currently licensed antibiotics evaluated for safety and efficacy.**

Based largely on clinical experience with radiation therapy, it is now known that organs such as the lung, kidney, and liver are sensitive to injury following radiation exposure. The mechanisms and extent of sub-clinical injury are not well understood. Such "inapparent" injuries may result in organ system complications later in life. For example, chronic renal failure has been observed in patients who have undergone radiation therapy, but the presumed damage to the renin-angiotensin system is not understood. Similarly, pneumonitis and fibrosis are complications of radiation therapy. Immediate post-exposure intervention may minimize some of these complications.

- **Mechanistic studies of radiation injury in organs such as the lung, kidney, and liver may lead to new strategies to prevent organ dysfunction.**

RECOMMENDATIONS FOR RESEARCH IN RADIOBIOLOGY

SHORT TERM (0-3 YEARS)

- The expansion of label indications for licensed products currently used to stimulate the production of hematopoietic cells in chemotherapy and radiotherapy patients should be pursued for use in radiological emergencies.

- The pharmacokinetics, safety, and therapeutic efficacy of antibiotics should be evaluated in validated animal models of radiation exposure.

- New scientific disciplines should be attracted to research in radiobiology, and increased numbers of new investigators should be trained in this field.

- Mechanisms to promote collaborations in radiobiology research among governmental organizations and institutions should be encouraged.

MID-TERM (3-5 YEARS)

- Biodosimetry tools, including molecular and cytogenetic assays that will result in tests with high sensitivity and specificity, should be developed. These tests should be automatic, rapid, inexpensive, and broad-based.

- The capacity to conduct longitudinal epidemiological studies to document long-term health effects following radiation exposure should be increased.

- Databases to assist in the analysis of exposure to radiation and resulting injuries, risk factors, and disease outcomes, should be established.

- Research on both the non-specific (e.g., oxidative reactions and stress, genetic disruption, differential expression of cytokines, alterations in protein expression, and tissue dysfunction) and chronic (e.g., delayed cellular and tissue injury, secondary fibrosis, and chronic inflammation) effects of radiation exposure should be encouraged.

- All relevant licensed drugs should be screened and evaluated for use in treatment of radiological injury.

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