

# Chapter 5

## THERAPY FOR BACTERIAL INFECTIONS FOLLOWING IONIZING RADIATION INJURY

THOMAS B. ELLIOTT, PhD,\* AND G. DAVID LEDNEY, PhD†

---

### INTRODUCTION

#### ETIOLOGY OF INFECTIONS

Endogenous Bacterial Infections

Exogenous Bacterial Infections

Fungal and Viral Infections

#### PROPHYLACTIC METHODS FOR PREVENTING INFECTIONS

#### THERAPEUTIC AGENTS

Antimicrobial Agents

Delivering Therapeutic Agents

Antimicrobial Agents Available for Managing Serious Infections After Irradiation

#### BIOLOGICAL RESPONSE MODIFIERS AND IMMUNOMODULATING AGENTS

#### COMBINED THERAPY: IMMUNOMODULATING AND ANTIMICROBIAL AGENTS

#### FUTURE CONSIDERATIONS

Drug Delivery

Antimicrobial Vaccines and Drugs

Immunomodulators

#### CURRENT RECOMMENDATIONS FOR MILITARY USE OR NATIONAL DISASTERS

#### SUMMARY

\*Research Microbiologist, Scientific Research Department, Radiation and Combined Injury Infection Group, Uniformed Services University of the Health Sciences, 8901 Wisconsin Avenue, Building 42, National Naval Medical Center, Bethesda, Maryland 20889

†Research Biologist, Scientific Research Department, Radiation and Combined Injury Infection Group, Uniformed Services University of the Health Sciences, 8901 Wisconsin Avenue, Building 42, National Naval Medical Center, Bethesda, Maryland 20889

## INTRODUCTION

Infectious diseases have historically caused more casualties than battle injuries. Nuclear, biological, and chemical weapons would cause a large number of casualties either during military operations or from terrorist events. Combinations of biological and nuclear weapons could be synergistic, so that injury severity would be much greater than from either weapons or infectious agents alone. Irradiation diminishes innate immune responses, particularly the inflammatory response, without which systemic infections among large numbers of casualties may become difficult to treat effectively and may not respond to antimicrobial regimens used in usual clinical practice. In many cases, partial-body irradiation could allow some undamaged hematopoietic and intestinal stem cells to repopulate those tissues; however, traumatic injury when combined with irradiation further complicates infection management. This chapter reviews the current state of knowledge since the last *Textbook of Military Medicine* on this topic and other presentations (Exhibit 5-1),<sup>1</sup> including laboratory investigations that illustrate essential principles and factors, about the available preventive and therapeutic measures against bacterial infections to decrease mortality and ameliorate synergistic combined insults, which are caused by endogenous and exogenous microorganisms and can occur during operations following exposure to ionizing radiation.

Factors that predispose individuals to irradiation-induced infections were described by Walker.<sup>1</sup> The

innate immune and inflammatory responses are impaired following irradiation. Numbers of circulating neutrophilic granulocytes as well as lymphocytes diminish and hematopoietic tissue (ie, bone marrow) is damaged by radiation. Neutropenia provides a valuable marker to indicate increased susceptibility to bacterial infections and is used as a clinical indicator to begin antimicrobial therapy.<sup>2-4</sup>

Bacterial infections are a major cause of morbidity and mortality in humans and laboratory animals that receive whole-body doses of ionizing radiation in a range that causes hematopoietic failure. Hematopoiesis and numbers of circulating blood leukocytes and thrombocytes are reduced within several days after irradiation.<sup>5</sup> Innate immune responses that protect against infection are therefore depressed<sup>1</sup> and hemorrhage occurs easily in tissues. The course of recovery from hematopoietic failure in laboratory animals differs from that seen in humans; the course of manifest illness in laboratory animals occurs within several days to a few weeks,<sup>6,7</sup> whereas the course in humans occurs between a few weeks to a few months following exposure.<sup>5</sup>

Fundamental research in radiation biology is essential and must be performed in laboratory animals because such studies may not be performed in humans. Prophylactic or therapeutic regimens must be evaluated in at least two suitable animal species (in place of a human clinical study) to satisfy the requirements of the US Food and Drug Administration (FDA). Laboratory animal models provide a scientific basis for comparing controlled variables of the complex cellular and molecular interactions of metabolism and immune responses in vivo.<sup>8</sup> They allow sufficient numbers of animals and trials to evaluate variables and ensure statistical validity. Results are generally reproducible and can often be extrapolated to humans.<sup>9</sup> The laboratory mouse provides a principal model for studies of infectious diseases and antimicrobial agents, including mechanism of action, pharmacokinetics, pharmacodynamics, efficacy, and toxicity.<sup>9,10</sup> The mouse mimics the human response to antimicrobial agents, although mice have a higher metabolic rate than humans. Andes and Craig<sup>11-13</sup> established guidelines for correlating efficacy between mice and humans. Further, the laboratory mouse is colonized by genera and species of intestinal facultative and anaerobic microorganisms similar to those found in humans.<sup>14-16</sup> The intestinal microbial ecosystem establishes and maintains functional stability despite constant challenges to the compositional sta-

### EXHIBIT 5-1

#### RECOMMENDED READING

Browne D, Weiss JF, MacVittie TJ, Pillai MV, eds. *Treatment of Radiation Injuries*. New York, NY: Plenum Press; 1990.

Ledney GD, Madonna GS, McChesney DG, Elliott TB, Brook I. Complications of combined injury: radiation damage and skin wound trauma in mouse models. In: Browne D, Weiss JF, MacVittie TJ, Pillai MV, eds. *Treatment of Radiation Injuries*. New York, NY: Plenum Press; 1990: 153-164.

Walker RI, Gruber DF, MacVittie TJ, Conklin JJ, eds. *The Pathophysiology of Combined Injury and Trauma: Radiation, Burn, and Trauma*. Baltimore, MD: University Park Press; 1985.

bility. Factors that challenge the microbial community include continuous turnover of the epithelium and mucus layer, a system that is open to the external environment, peristaltic activity that ensures constant exposure to dietary macromolecules, gastrointestinal secretions, and exogenous bacteria. The irradiated mouse provides a unique model for testing safety, efficacy, and immunogenicity of potential therapeutic drugs in immunodepressed animals because effects of irradiation are prolonged compared to only a few days following drug-induced immunosuppression.

Neutropenia and thrombocytopenia have long been recognized as significant complications and risk factors of serious infections, particularly sepsis, following irradiation. Neutropenia is used as an indicator for initiating antimicrobial therapy, whereas thrombocytopenia was shown to be an independent prognostic indicator of mortality only in patients with sepsis in an intensive care unit.<sup>17</sup> In irradiated laboratory animals (eg, mice), circulating leukocytes drop precipitously within 2 days to barely detectable numbers, begin to recover gradually after approximately 15 days, and approach normal levels in 28 days or longer.<sup>7</sup> The number of thrombocytes in mice decreases after 5 days and begins to recover within 10 to 12 days. In humans, lymphocytes decrease promptly, neutrophils decline over several days, thrombocytes begin to decrease after approximately 8 days, and hematopoiesis begins to recover after 30 days.<sup>5</sup>

Profound neutropenia ( $< 1.0 \times 10^5$  neutrophilic granulocytes/mL, or 100 neutrophils/ $\mu$ L), particularly if the duration is more than 7 days, is the greatest risk factor for infection. Other factors that will affect the efficacy of treatment include phagocytic and bactericidal function of granulocytes and macrophages, changes in the endogenous microbial flora, endemic microorganisms in the local environment, changes in defensive barriers, and general health status (for example, combat personnel are likely to be nutritionally and physically stressed). Secondary fungal infections could also occur as the duration of neutropenia increases.

Since the end of the Cold War in the early 1990s, concerns about nuclear disasters have not diminished; rather they have shifted to emphasize the low-dose acute and low-dose-rate chronic irradiation scenarios of nuclear accidents, tactical situations, and terrorist activities. During 1995–1996, a North Atlantic Treaty Organization working group considered the range of gamma photon radiation between 0.25 and 1.5 Gy acceptable for conducting military operations. The US Army Groundfire 95 Low Level Radiation Exposure Issues Workshop examined options for soldiers deployed as part of peacekeeping or humanitarian

assistance missions. The dose range of radiation between 0.70 and 3.0 Gy was considered to produce effects of immediate military relevance. Significant risk was acknowledged to range from 0.25 to 0.70 Gy. The North Atlantic Treaty Organization standardization agreement 2083, Commander's Guide on the Effects from Nuclear Radiation Exposure During War, states the doses and probable tactical effects on groups. A dose of 0.75 Gy up to 1.25 Gy will induce probable initial tactical effects up to 5% latent ineffectiveness, which is "the casualty criterion defined as the lowest dose at which personnel will (a) become combat ineffective (less than 25% capable) at any time within 6 weeks post exposure followed by death or recovery, or (b) become performance degraded (ie, 25–75% capable) within 3 hours after exposure and remain so until death or recovery."<sup>18</sup> An exposed group would be considered combat effective and would not require medical care after 1 day following a dose ranging from 0.75 to 1.25 Gy. However, following doses greater than 1.25 Gy, groups probably would not be able to perform complex tasks and sustained efforts would be hampered.

Such radiation doses are not associated with neutron irradiation, which occurs during detonation of a nuclear weapon. In the latter case, those persons who would be in the zone of survivability from the heat and blast effects would be exposed to neutrons as well as gamma photons. The approximate  $LD_{50/30}$  (the dose of radiation required to kill 50% of the test population within 30 days) in humans for uncomplicated prompt irradiation is generally accepted as approximately 4.5 Gy gamma photons with basic clinical support or 3.0 Gy without clinical support, whereas the  $LD_{50/30}$  in B6D2F<sub>1</sub>/J female mice in our laboratory in the Armed Forces Radiobiology Research Institute is 9.4 Gy, or approximately twice the human value. The  $LD_{50}$  (median lethal dose) decreases with combined injury, and the  $LD_{50}$  increases as the dose rate decreases, such as in a fallout field.<sup>19</sup>

Lethal doses of ionizing radiation induce systemic infections that are caused by endogenous or exogenous microorganisms. Endogenous infections arise from facultative microorganisms that translocate from the upper and lower intestinal tract, which is normally colonized predominantly by anaerobic bacteria and lesser numbers of facultative bacteria. The anaerobic bacteria ordinarily provide colonization resistance against pathogenic exogenous microorganisms. On the other hand, nonlethal doses of ionizing radiation enhance susceptibility to exogenous bacterial infections acquired from the environment and enhance mortality, as well.

## ETIOLOGY OF INFECTIONS

A predominant number of casualties in Hiroshima and Nagasaki in August 1945 who were beyond the range for blast and heat injuries and who suffered gamma radiation injury with associated fever and pronounced leukopenia developed overwhelming infections that became septicemias. Estimates of the mortality rate from irradiation varied widely in subsequent reports of those two major events. Representative autopsy reports of victims following the atomic bombings showed that oropharyngitis was most frequently seen among the various infections, with necrotizing tonsillitis in a majority of cases, followed by infections of the large intestinal tissue, esophagus, bronchus, lungs, uterus, and urinary tract. These sites were considered the portal of entry for generalized infection (ie, septicemia and bacteremia, in many cases).<sup>20</sup> However, no definitive incidence or specific causes of infection were described. Following the accident at the Chernobyl, Ukraine, power station on April 26, 1986, 500 individuals were hospitalized. Over 100 received doses of radiation greater than 1 Gy. Over 90% of hospitalized victims survived. Although reports did not provide incidence of, causes of, or specific therapy for infections during the first month after the event, *Staphylococcus* species were reported to be the most frequent cause of septicemias.<sup>21</sup> Antimicrobial selective decontamination of the intestinal flora with sulfamethoxazole/trimethoprim and nystatin was used to reduce the chance of infection. Systemic antimicrobial therapy with two aminoglycosides, three cephalosporins, or two semisynthetic penicillins was used in febrile, granulocytopenic patients. Antifungal amphotericin B was used when fever persisted for more than 1 week, and antiviral acyclovir was used when herpes simplex virus was activated.

The kinds of microorganisms that cause infections depend on the quality and dose of radiation in each case. Gram-positive, nonsporulating rods and enterococci tend to predominate in the ileum of laboratory mice that are given moderately lethal doses (10–12 Gy) of gamma photon radiation, whereas facultative gram-negative rods of the family Enterobacteriaceae predominate in mice that are given equivalent lethal doses of mixed-field (gamma and neutron) radiation (6–7 Gy, where the ratio of neutron dose to total dose of neutrons plus gamma photons is 0.67).<sup>22,23</sup> Polymicrobial septicemias occur after lethal doses of radiation. The recovery of bacteria from the blood of mice correlated to changes that occurred in the gastrointestinal flora following exposure to ionizing radiations. Following lethal doses of gamma radiation (10 Gy), numbers of facultative and anaerobic bacteria in the ileum of

experimental C3HeB/FeJ mice decreased beginning 2 days after irradiation.<sup>24</sup> This decline reached a nadir between 5 and 7 days after irradiation. Mortality began 7 to 9 days after irradiation and correlated with an increase in the number of Enterobacteriaceae in the intestinal flora, while the numbers of anaerobic bacteria remained low. Endogenous *Escherichia coli* and *Proteus mirabilis* appeared in the blood, spleens, and livers of the animals. Anaerobic bacteria, which comprise approximately 90% of intestinal microflora, provide colonization resistance against invading facultative bacteria. With decreased numbers of anaerobic bacteria following lethal doses of radiation, the facultative bacteria, including Enterobacteriaceae, have the opportunity to fill the niche normally filled by the anaerobic bacteria and then, when intestinal tissue is injured by radiation, the bacteria translocate through the lymphatics into the blood. It appears likely that the selective translocation of bacteria is related to the greater injury to intestinal tissues by neutrons compared with injury caused by gamma photons based on the detailed findings of Lawrence and Tennant, who compared injuries with neutrons and X-rays.<sup>25</sup> Predominantly gram-negative sepsis followed lethal mixed-field ( $n/[n+\gamma] = 0.67$ ) irradiation, whereas predominantly gram-positive sepsis followed lethal <sup>60</sup>Co-gamma-photon irradiation in mice.<sup>26</sup>

Wound and burn infections are more severe in the irradiated than in the nonirradiated host. The number of organisms in these infections is greater than in a nonirradiated host, and antimicrobial agents have a limited role in preventing systemic complications. In laboratory animals, wounds or burns that are infected with exogenous bacteria develop life-threatening infections after nonlethal irradiation, even with antimicrobial therapy.<sup>27</sup> Polymicrobial infections are common, as demonstrated by the findings of the following experiment. In 1957, swine were placed behind sheets of glass at measured distances from ground zero of a nuclear detonation, including at 4,430 ft (Station 6), 4,770 ft (Station 7), and 5,320 ft (Station 8).<sup>28</sup> Bacteria were isolated from wound, blood, and fecal specimens from each animal at these three stations. The number of wound cultures that demonstrated growth of bacteria decreased with time on day 1 (44%), day 2 (22%), day 3 (11%), and day 5 or later (4%). A greater number of “aerobic gram-positive” microorganisms were isolated ( $10^4$  to  $> 10^6$ ), which increased with time, than “coli-form” bacteria ( $< 10^3$  to  $10^5$ ), which decreased with time. The predominant bacteria isolated from wounds included *Micrococcus pyogenes* var *albus* (*Staphylococcus epidermidis*), *M pyogenes* var *aureus* (*Staphylococcus*

*aureus*),  $\beta$ -hemolytic *Streptococcus* (several species of *Streptococcus* are known to produce complete hemolysis of red blood cells in culture media), "coliforms" (lactose-fermenting Enterobacteriaceae, such as *Escherichia* species, *Enterobacter* species, *Klebsiella* species, etc), *Streptococcus faecalis* (*Enterococcus faecalis*), and  $\alpha$ -hemolytic *Streptococcus* (numerous species). *Clostridium* species and *Proteus vulgaris* appeared in low numbers of wounds. The predominant microorganism found in blood cultures was *Staphylococcus albus* (*S. epidermidis*) at the three stations.

Consequently, the choice of antimicrobial agents depends on the quality and dose of radiation and the microorganisms that cause the ensuing infection. Prompt laboratory identification of the microorganisms that cause the infection is imperative to ensure effectiveness of the carefully selected therapeutic agents. Early isolation and identification of resident microorganisms with antimicrobial susceptibility assessment from the oropharynx, rectum, and axilla of casualties would be valuable to compare with those isolated later, when casualties develop subsequent systemic infection, and to provide optimal antimicrobial therapy based on the pharmacodynamic parameters of the selected drugs. Such a preliminary study was performed in nonhuman primates (*Macaca mulatta*) before irradiation<sup>29</sup> in preparation for subsequent studies after irradiation. Knowledge of predominant endemic microorganisms in a geographical region where a conflict could occur would also aid in planning appropriate treatments.

## Endogenous Bacterial Infections

### Sepsis

Following lethal doses of radiation, sepsis is a complex consequence of depressed hematopoiesis, immunosuppression, and mucosal damage, as well as injury to cells of the intestines and lungs. Bacteria translocate principally from the intestinal lumen but also from other mucous membranes or wounds into local and regional lymphatics, causing sepsis, multiple organ failure, and death. Following nonlethal irradiation, susceptibility to exogenous infection is increased, which can progress to sepsis, but bacterial translocation from endogenous sources does not generally occur after sublethal irradiation. Trauma and physical exercise stress also increase bacterial translocation from the intestines and could contribute to increased infections after nonlethal irradiation. Sepsis is characterized by uncontrolled host inflammatory responses to bacterial infection, including overproduction of proinflammatory cytokines as the syndrome progresses toward multiple organ failure.<sup>30,31</sup> Despite improved

antimicrobial agents and clinical support, mortality from sepsis in intensive care units has remained at 35% to 45% for more than a half century.<sup>32</sup>

In clinical practice, severe systemic infections caused by gram-negative bacteria are generally treated with aminoglycosides combined with  $\beta$ -lactam antibiotics. From 1989 until recently, vancomycin was reserved to treat severe infections caused by antibiotic-resistant, gram-positive bacteria in an immunocompromised host. Effective therapy can be provided by single agents, including piperacillin/tazobactam, carbapenems or fourth-generation cephalosporins, or a combination of penicillin and gentamicin with or without vancomycin, depending on the microorganisms that cause the specific infection.

Selective decontamination of the digestive tract with antimicrobial agents is an infection-control strategy that can prevent infection in an immunocompromised host.<sup>33</sup> Four objectives of selective decontamination using four component protocols (compared to conventional therapy for sepsis)<sup>34</sup> include the following: (1) treat the primary endogenous infection with a systemic parenteral antimicrobial agent; (2) prevent a secondary endogenous infection by microorganisms acquired during hospitalization with enteral, non-absorbable agents; (3) prevent exogenous infection through a rigorous hygiene protocol; and (4) perform surveillance cultures of the intestinal tract to detect potential exogenous microorganisms that have been acquired. This evidence-based medical intervention significantly reduces morbidity and mortality, prevents the emergence of resistant microorganisms, and is cost effective.<sup>35</sup>

Following irradiation, the concept of selective decontamination can be adapted, but the antimicrobial agents are chosen to inhibit Enterobacteriaceae and spare the indigenous anaerobic bacteria. 4-Fluoroquinolones possess high bactericidal activity against most gram-negative bacteria in vitro.<sup>36</sup> These agents can be given orally, are relatively free of serious side effects, and are used for selective decontamination of the intestinal tract to prevent sepsis in neutropenic, immunocompromised hosts. Except for norfloxacin, the quinolones are readily absorbed, so not only do they reduce the number of facultative enteric bacteria in the intestinal lumen without suppressing anaerobic bacteria, they also eliminate facultative microorganisms that might spread systemically. Other agents that are nonabsorbable include polymyxin B and neomycin. These agents are useful for selective decontamination, but they would be toxic if they were absorbed through injured intestinal mucosa.

To be optimal, selective decontamination should be initiated to anticipate and prevent the translocation

of intestinal bacteria following irradiation. Time of initiation of selective decontamination, as for systemic antimicrobial therapy, is not definitively established but will depend on timing of thrombocytopenia and neutropenia as measurable indicators of susceptibility, dose and quality of radiation, source and extent of infection, and the extent of trauma, burns, or other physical injuries.

### Polymicrobial Infections

Polymicrobial sepsis following lethal doses of ionizing radiation or wound infections can occur following irradiation that is associated with trauma. Such infections are enhanced because of depressed innate immune responses and translocation from the intestinal tract.

Effective management of polymicrobial infections in an irradiated host is complex.<sup>37</sup> It is imperative to prevent translocation of intestinal bacteria that cause sepsis, multiple organ failure, and death. Translocation of facultative and aerobic intestinal bacteria can be increased by suppressing the indigenous anaerobic intestinal flora, which normally provides colonization resistance against potential invading bacteria, particularly gram-negative aerobic and facultative microorganisms.<sup>24</sup> Effective therapy can be achieved by using antimicrobial agents, which eliminate the microorganisms that cause the local or systemic infection and yet possess minimal inhibitory activity against strictly anaerobic bacteria in the intestinal tract.<sup>38</sup> For example, in experimentally irradiated mice, metronidazole enhanced mortality because it reduced the anaerobic intestinal bacteria. On one hand, successful management of intraabdominal and other polymicrobial infections (eg, *E coli* and *Bacteroides fragilis*) requires administration of antimicrobial agents that are effective against both microorganisms.<sup>39</sup> However, in irradiated hosts, adverse effects can be associated with the use of an antimicrobial agent that is effective against anaerobic bacteria. When the numbers of intestinal anaerobic bacteria are reduced, Enterobacteriaceae may increase in number, translocate, and cause sepsis.<sup>24,40</sup>

Ofloxacin and metronidazole efficacy was evaluated in mice given 8.0 or 8.5 Gy <sup>60</sup>Co gamma-photon radiation<sup>40</sup> (levofloxacin is the active racemic isomer of ofloxacin). Metronidazole (50 mg/kg) or ofloxacin (40 mg/kg), administered intramuscularly in divided doses every 12 hours, was initiated 48 hours after irradiation for 21 days. After 8.0 Gy, 40% of saline-control and 90% of ofloxacin-treated mice survived at 30 days, whereas no mice treated with metronidazole survived after 16 days ( $P < 0.05$ ). After 8.5 Gy, all saline-treated control mice were dead by day 25 but all metronida-

zole-treated mice were dead by day 9 ( $P < 0.05$ ), and 50% of ofloxacin-treated mice survived 30 days.

Use of clindamycin may also have similar adverse effects following irradiation. Clindamycin is active against most aerobic and anaerobic gram-positive cocci as well as gram-negative anaerobic bacilli, but has poor activity against most gram-negative facultative aerobes and *Enterococcus* strains.<sup>41</sup> In sublethally irradiated experimental mice that were challenged on day 4 with *Bacillus anthracis* Sterne spores intratracheally, a polymicrobial sepsis ensued.<sup>42</sup> Twice-daily, subcutaneous antimicrobial therapy with ciprofloxacin (50 mg/kg), clindamycin (200 mg/kg), or a combination was started 24 hours after bacterial challenge and continued for 21 days.<sup>43</sup> Ciprofloxacin and clindamycin separately improved 45-day survival 77% and 86% ( $P < 0.001$ ), respectively, compared to saline-treated controls (4%), but combination therapy decreased survival to 45% compared to clindamycin alone ( $P < 0.01$ ). In this study, the combination of clindamycin and ciprofloxacin was used to broaden the antimicrobial spectrum of therapy and increase survival. An earlier study in healthy human volunteers did not detect adverse interactions between ciprofloxacin and clindamycin, nor any changes in the pharmacokinetics of ciprofloxacin when combined with clindamycin.<sup>44</sup> This approach, however, revealed an adverse interaction between clindamycin and ciprofloxacin, particularly in gamma-irradiated animals, that reduced survival significantly.

### Exogenous Bacterial Infections

Nonlethal doses of ionizing radiation sufficiently depress innate immune responses to increase susceptibility to exogenous bacterial infections, so that small numbers of bacteria can cause an enhanced infection that becomes life threatening,<sup>7,8,42,45</sup> as seen in human patients who are given whole-body radiation treatment prior to bone marrow transplantation.<sup>46</sup> During the 2- to 4-week period of profound neutropenia after irradiation, sources of infection include the patient's own microflora, particularly *S epidermidis*. Microorganisms, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, are frequently isolated in immunocompromised patients or those who have been exposed to ionizing radiation (therapeutically or accidentally). Known microorganisms are used as indicators to demonstrate susceptibility and to evaluate essential factors of effective therapy, including route of infection, route of administration of antimicrobial agents, and duration of therapy.

When *K pneumoniae* is inoculated subcutaneously into experimental mice 3 or 4 days after nonlethal irradiation, the bacterial LD<sub>50/30</sub> decreases from ap-

proximately  $4 \times 10^6$  colony-forming units (CFUs) in nonirradiated animals to  $2 \times 10^2$  CFUs in irradiated mice that are given 7.0 Gy gamma photons ( $\gamma$ ) or 3.5 Gy mixed-field neutrons (n) and gamma ( $\gamma$ ) radiation [ $\gamma/(n+\gamma) = 0.64$ ].<sup>45,47</sup> These are nonlethal doses of radiation in mice. When ceftriaxone was started 1 day after bacterial challenge and given subcutaneously once daily for 10 days, 60% to 70% of mice survived.<sup>48</sup>

Similarly, when  $10^8$  CFUs *K pneumoniae* were inoculated per os into mice 2 days after 8.0 Gy <sup>60</sup>Co gamma-photon radiation, the quinolones (ofloxacin, pefloxacin, and ciprofloxacin), when given orally starting 1 day after bacterial challenge, reduced colonization of the ileum from 57% in controls to 13% in treated animals ( $P < 0.005$ ).<sup>38</sup> Survival increased from 25% in controls to 70% to 85% in treated mice (keeping in mind that levofloxacin is the active racemic isomer of ofloxacin). When  $10^7$  CFUs *P aeruginosa* were inoculated per os, oral ofloxacin reduced colonization of the ileum from 86% to 17% and survival increased from 20% in controls to 95% in treated animals ( $P < 0.005$ ).<sup>38,49,50</sup>

Duration of antimicrobial therapy and eradication of infection are other factors to consider in the irradiated host. In one laboratory study, mice were given 8.0 Gy <sup>60</sup>Co gamma-photon radiation.<sup>51</sup> A dose of  $10^8$  CFUs *K pneumoniae* was given orally 4 days after irradiation and therapy with ofloxacin was started 1 day later. One group of 20 mice was given ofloxacin for 7 days,

one group for 21 days, and one group was untreated. The optimal duration of therapy with ofloxacin for *K pneumoniae* infection was found to be 21 days (90% survival), compared to 7 days (55% survival). On the fourteenth day after irradiation, *K pneumoniae* was isolated in the ileum of 7 of 9 mice that had received ofloxacin for 7 days and 5 of 6 untreated mice, but no *K pneumoniae* was found in the ileum of mice that were treated for 9 days with ofloxacin ( $P < 0.05$ ). Also, *K pneumoniae* was isolated from the livers of 4 of 6 untreated mice, in 4 of 9 that had received 7 days of ofloxacin, and in none of the mice that had received 9 days of ofloxacin ( $P < 0.05$ ). A 21-day course of therapy would provide protection in humans during the period of greatest risk for infection until the innate immune responses recover and circulating neutrophilic granulocytes begin to approach normal numbers.<sup>51,52</sup>

### Fungal and Viral Infections

In addition to bacterial infections, it is important to be aware that fungi may cause life-threatening infection if leukopenia is prolonged after irradiation for more than 14 days. Further, herpes simplex virus was activated in many victims of the Chernobyl accident. The viral infections responded well to acyclovir. However, appropriate preventive and therapeutic approaches to fungal and viral infections are beyond the scope of this chapter.

## PROPHYLACTIC METHODS FOR PREVENTING INFECTIONS

Primary or recent booster vaccinations against common endemic and epidemic infectious agents are likely to continue providing protection if immunity relies on circulating antibodies. Vaccinations that induce cell-mediated responses by lymphocytes and that are administered before irradiation, however, may provide inadequate immunity following even low, nonlethal doses of ionizing radiation because the number of circulating lymphocytes decreases rapidly. Further, recovery of the number of lymphocytes is likely to be too slow to respond adequately to a vaccine. Because the number of lymphocytes is diminished after irradiation, any vaccination soon after irradiation is unlikely to provide adequate immunity. In particular, the use of live, active, or attenuated vaccines is contraindicated because these agents could induce life-threatening infection if given within a few weeks before or soon after exposure to ionizing radiation.

Methods that prevent exposure to potential pathogenic microorganisms should be initiated early in the care of medical casualties who have received moderate to severe doses of ionizing radiation, whether

in the field or hospital. Such methods include disinfecting water, appropriately cooking foods, frequent hand washing, use of medical or dental gloves, and air filtration. Selective decontamination of aerobic and facultative intestinal bacteria, while preserving anaerobic flora, can reduce bacterial translocation of facultative and aerobic bacteria, a major source of endogenous bacterial infections. Puncturing skin with needles or using intravenous catheters should be avoided, if possible, because bacteria can be inserted into the injection site and microbial biofilms can easily develop on catheters in situ and become sources of infection. Alimentary feeding will stimulate and maintain the integrity of the intestinal tract as well as provide adequate nutrition. The ingestion of probiotics that are selected species and strains of microorganisms, particularly *Lactobacillus* species used for preparing food products (eg, yogurt), may help prevent endemic gastrointestinal infections, but scientific evaluations are limited and need further investigation to substantiate their efficacy and lack of virulence in immunocompromised hosts.

## THERAPEUTIC AGENTS

Antimicrobial agents are the mainstay for therapeutic management of bacterial infections. Cidal antimicrobial agents rather than inhibitory agents offer the best treatment after irradiation because of decreased innate immune responses, which are required for efficacy of inhibitory, or bacteriostatic, antimicrobial agents. Nonspecific and specific immunomodulatory agents also show propensity to improve the outcome of infections after irradiation, but such substances remain under investigation. Use of single agents, either an antimicrobial or an immunomodulator, is not likely to provide effective therapy after irradiation, based on experimental evidence. Prompt therapeutic interventions that would enhance the innate immune responses in a natural manner as well as eliminate pathogenic microorganisms would be a critical requirement to improve chances of survival from infections after irradiation.

### Antimicrobial Agents

Careful selection of antimicrobial agents depends on knowledge of both quality and dose of ionizing radiation as well as antimicrobial susceptibility of the microorganisms that cause infection, particularly for polymicrobial sepsis. A radiation event becomes a distinct milestone for measuring time of onset of signs and symptoms and initiation of therapeutic modalities. Time of initiation and duration of antimicrobial therapy depend on the timing of thrombocytopenia and neutropenia as measurable indicators of susceptibility, as well as on dose and quality of radiation, source and extent of infection, and the extent of trauma, burns, or other physical injuries. Experimentally irradiated animals require antimicrobial support during the period when they are most vulnerable to polymicrobial infection (between 7 and 25 days after irradiation, or between approximately 2 and 25 days after combined injury). Inappropriate choice of antimicrobial agents and dosage can lead to failure to eradicate the infection. Bacterial resistance and adverse effects of therapeutic agents can complicate therapy even further.

The dose and interval of administration for each antimicrobial agent depend on the particular pharmacokinetics and pharmacodynamics. The pharmacokinetic and pharmacodynamic parameters are unique for each antimicrobial agent and may vary following irradiation compared with those in nonirradiated patients, perhaps even more so following combined injury. Consequently, antimicrobial dosage regimens that would be appropriate and effective in nonirradiated persons may need to be adjusted to achieve a satisfactory outcome in ir-

radiated persons based on preliminary research data from laboratory animals (Elliott TB, unpublished data). The concentration of the selected antimicrobial agent at the site of infection should exceed the minimum inhibitory concentration for the specific microorganisms for at least 40% of the dosing interval for  $\beta$ -lactam antibiotics.<sup>13</sup> For aminoglycosides and quinolones, the maximum concentration or the area under the concentration-time curve relative to the minimum inhibitory concentration predicts their efficacy.

High doses of one or more broad-spectrum antimicrobial agents should be continued until the number of neutrophilic granulocytes has recovered to at least  $5.0 \times 10^8$  cells/L (500 cells/ $\mu$ L) and the patient is afebrile for 24 hours.<sup>2-4,36,53</sup> Aminoglycosides should be avoided because of toxicities associated with this class of agents. Regimen adjustments should be based on specific laboratory findings, including identification of microbial species and antimicrobial susceptibilities. When antimicrobial-resistant, gram-positive bacteria, such as *Enterococcus* species, are isolated from a patient, vancomycin, linezolid, or daptomycin should be included in the regimen.

Alternative dosing regimens are designed and adjusted based on pharmacokinetic parameters to improve eradication of infection and clinical outcome. The desired microbiological outcome is indicated by eradication or prevention of infection and the desired clinical outcome is survival or, at least, extension of survival time to allow time for additional interventions to enhance recovery of radiation-injured proliferative tissues. Evaluation of pharmacokinetics and pharmacodynamics provides a basis for developing alternative strategies to achieve a successful microbiological outcome for radiation-induced sepsis. Adjusting some of the variable principal factors of traditional dosing regimens (dose, route, duration, frequency of administration, period of greatest risk, emergence of antimicrobial-resistant microorganisms, and combination therapy) could improve survival outcome as well.<sup>54</sup> Particularly for concentration-dependent agents, increasing dose and dose rate would likely improve the rate of bacteria elimination; however, that adjustment alone will not suffice following lethal irradiation. The duration of treatment could be limited to the period of greatest risk. Frequent drug administration further irritates radiation-injured soft tissues and can cause inadvertent bleeding because of thrombocytopenia. Therefore, reducing administration frequency to once daily would alleviate intermittent injury to soft tissues. Since efficacy of the quinolones is concentration-dependent, a higher dose once daily

might improve survival as well as eradicate infection.<sup>55</sup> This latter strategy might also alleviate some of the adverse consequences of administering aminoglycosides parenterally following irradiation, since aminoglycosides have been shown to provide efficacy when given once daily for serious infections in neutropenic patients,<sup>55,56</sup> but should be based on pharmacodynamic end points and individualized pharmacokinetic assessment in critically ill surgical patients.<sup>57</sup>

## Delivering Therapeutic Agents

### Oral

Oral administration of therapeutic agents is optimal and preferred because this route avoids local bleeding and introducing bacteria at the injection site, is non-invasive, and is especially practical for treating large numbers of casualties. However, using this route of administration depends on the patient's ability to tolerate oral administration (irradiation can induce emesis), the presence of intestinal motility, and absorption of the selected drug from the intestine, which might be altered by irradiation, depending on the quality or dose of radiation. Drugs administered by this route can alter the composition of the intestinal microflora, so oral administration should be used with caution. Oral delivery might be more appropriate in persons who receive low doses of radiation that would cause minimum disturbance to intestinal tissue.

### Intravenous

The intravenous route provides immediate, therapeutic concentrations of antimicrobial agents systemically. An intravenous injection could introduce skin or environmental microorganisms directly into the blood, which could be dangerous because of reduced host resistance to infections after irradiation. Because microorganisms form a biofilm on intravenous catheters, which remain in situ for several days, the biofilm provides a nidus for infection. Intravenous delivery might be more appropriate for those who receive high, lethal doses of radiation that cause injury to intestinal tissue and alter absorption of oral drugs. Strict aseptic maintenance at the insertion site of an intravenous catheter is required to prevent a local infection that could become systemic and local bleeding because of thrombocytopenia.

### Subcutaneous

The subcutaneous route also provides direct introduction of therapeutic agents, but absorption depends on adequate local circulation in capillaries.

Subcutaneous drug administration could be deleterious because multiple daily injections are required that would introduce skin or environmental microorganisms into those who have reduced resistance to infection after irradiation. Further, subcutaneous bleeding can occur because of thrombocytopenia. Survival was lower in experimental, sublethally irradiated mice that were given only daily injections of saline or water as a control vehicle than in mice that were given no injections after irradiation.<sup>51</sup> However, this risk can be reduced by cleaning injection sites with iodine solution and rinsing with 70% ethanol three times before injection.

### Intramuscular

Similar to the subcutaneous route, the intramuscular route depends on adequate local circulation but is contraindicated because excessive bleeding is a major consequence due to thrombocytopenia.

### Topical

Antimicrobial salves or lotions have been shown to reduce mortality from infections in experimental irradiated animals that have sustained combined injury from burns or wounds.<sup>58,59</sup> Although local absorption may provide sufficient therapeutic concentrations in injured tissues, absorption is inadequate to achieve therapeutic concentrations systemically and in deep tissue.

## Antimicrobial Agents Available for Managing Serious Infections After Irradiation

Three important principles were established in a mouse model that impact efficacious antimicrobial therapy after radiation exposure. First, irradiation increases the probability of translocation of intestinal bacteria.<sup>23</sup> Second, the management of a polymicrobial infection after lethal irradiation is complex and requires the use of antimicrobial agents effective against both gram-positive and gram-negative facultative bacteria, which readily develop antimicrobial resistance.<sup>36</sup> Third, killing anaerobic intestinal bacteria, which are required to maintain colonization resistance against pathogenic bacteria,<sup>33</sup> enhances mortality after lethal irradiation.<sup>24</sup> Further, bactericidal activity is required against infections after irradiation. Bacteristatic agents require phagocytic cells, including granulocytes and macrophages, to be effective against microorganisms; however, the innate immune responses are greatly diminished within a few days after irradiation. The fundamental principles for selecting the most appropriate antimicrobial agents in the following classes

have been recommended for managing post-irradiation infection.<sup>36</sup> Conventional dosing regimens for antimicrobial agents are readily available<sup>60</sup> but some dosage regimens should be adjusted for irradiated neutropenic casualties.

### Penicillins

Penicillin G remains the drug of choice to control many microorganisms that do not produce  $\beta$ -lactamases, including streptococci, community isolates of *S aureus*, and nonresistant, anaerobic, gram-negative bacilli. However, penicillin G would not be used in neutropenic patients because of the prevalence of antimicrobial resistance among microorganisms in the general population. A  $\beta$ -lactamase-producing strain could "shield" penicillin-susceptible microorganisms from the antibacterial activity of penicillin in a mixed infection. Combinations of a penicillin-class agent plus a  $\beta$ -lactamase inhibitor can provide effective therapy against some penicillin-resistant bacteria.

### Cephalosporins

Cephalosporin activity against aerobic and facultative gram-positive and gram-negative bacteria and *Bacteroides* species varies. Cefoxitin, a second-generation cephalosporin, is the most active cephalosporin against  $\beta$ -lactamase-producing *Bacteroides* species. Third-generation cephalosporins have improved activity against the family Enterobacteriaceae. Ceftazidime and cefepime are cephalosporins that are effective against *P aeruginosa*. However, extended spectrum  $\beta$ -lactamases confer resistance among microorganisms against extended-spectrum cephalosporins and monobactams. Consequently, their use in neutropenic patients is limited.

### Carbapenems

Carbapenems are bactericidal against methicillin-resistant staphylococci and are resistant to most  $\beta$ -lactamases. Imipenem, a thienamycin antibiotic, coupled with cilastatin, and meropenem have a broad spectrum of antibacterial activity against strictly anaerobic bacteria and gram-negative and gram-positive facultative bacteria.

### Metronidazole

This synthetic antimicrobial agent has exceptional activity against anaerobic bacteria only. Metronidazole should be used with caution after irradiation because its activity can be deleterious in an immunocompro-

mised host. Metronidazole suppresses the indigenous intestinal anaerobic flora following irradiation so that Enterobacteriaceae can grow unimpeded, translocate from the intestinal tract, and cause sepsis.<sup>24</sup>

### Clindamycin

This semisynthetic antibiotic has a broad spectrum of activity against anaerobic bacteria and gram-positive cocci. It also inhibits production of toxins by *Clostridium* species and *Streptococcus pyogenes*. It is indicated in the therapy of serious infections caused by *S pyogenes* as well as anaerobic bacteria.

### Aminoglycosides

This class of antibiotics is the major means of controlling gram-negative enteric bacterial infections. These drugs are bactericidal against gram-negative bacilli. However, many gram-negative enteric bacteria exhibit resistance to aminoglycosides. Use of gentamicin, tobramycin, and amikacin is limited because of nephrotoxicity and ototoxicity. They are not effective against anaerobic bacteria or aerobic bacteria in an anaerobic environment.

### Quinolones

The 4-fluoroquinolones are active against Enterobacteriaceae, *S aureus*, and other facultative and aerobic bacteria. They are bactericidal and primarily used to inhibit gram-negative bacteria. Ciprofloxacin is the most effective agent in this class against *P aeruginosa*. With increasing use of quinolones, resistance of *P aeruginosa* and Enterobacteriaceae has increased against these drugs. Ciprofloxacin and levofloxacin are included in the Strategic National Stockpile for use against infections following mass casualty events. Newer quinolones, such as moxifloxacin and gatifloxacin, are effective against gram-positive facultative cocci, including *Streptococcus pneumoniae*, as well as gram-negative bacilli. Ciprofloxacin and moxifloxacin were shown to ameliorate leukopenia and neutropenia in immunocompromised mice.<sup>61</sup> The quinolones are not as toxic as aminoglycosides and the older members do not inhibit anaerobic bacteria. However, one quinolone, pefloxacin, was found to decrease bone marrow progenitor cells and overall survival in nonlethally irradiated mice.<sup>62</sup>

### Vancomycin

After 1989, vancomycin was reserved as the treatment of last resort for infections caused by antimicrobial-resistant, gram-positive bacteria, especially

*Enterococcus faecium*, *S aureus*, and *S pneumoniae*. However, resistance to vancomycin has emerged in recent years. This drug is bactericidal against gram-positive bacteria. Recommendations for use of vancomycin can be found in the current literature.<sup>2,63</sup>

### Oxazolidinones

The oxazolidinones are represented by linezolid, the first member of this class of synthetic drugs to be approved by the FDA in April 2000. Linezolid is reserved for treating infections associated with vancomycin-resistant *E faecium*, including bloodstream infection, hospital-acquired pneumonia, and complicated skin and skin structure infections, including cases due to methicillin-resistant *S aureus*. In addition, this drug may be used to treat community-acquired pneumonia and uncomplicated skin and skin structure infections. Linezolid is bacteriostatic against enterococci and staphylococci. It is bactericidal against the majority of strains of streptococci.

### Streptogramins

Quinupristin and dalfopristin are two semisynthetic derivatives of pristinamycin I and IIa, respectively. They are combined in a single formulation that is indicated for the treatment of patients with serious or life-threatening infections associated with vancomycin-resistant *E faecium* bacteremia or complicated skin and skin structure infections caused by *S aureus* (methicillin susceptible) or *S pyogenes*. Quinupristin/dalfopristin formulation is bacteriostatic against *E faecium* but not *E faecalis*, and bactericidal against strains of methicillin-susceptible staphylococci. Because it is bacteriostatic against *E faecium*, phagocytic leukocytes are required for clearance. Therefore, this drug may not be effective against *E faecium* in individuals who are neutropenic.

### Synergistic Combinations of Antimicrobial Agents

To be effective in an immunocompromised host, antimicrobial agents must be bactericidal because the innate immune response is not reliable for clearing infectious agents. Synergism has been demonstrated with aminoglycosides together with penicillin or vancomycin against enterococci,  $\alpha$ -hemolytic streptococci, and *Prevotella melaninogenica* (*Bacteroides melaninogenicus*); nafcillin against *S aureus*; ticarcillin against *P aeruginosa*; cephalosporins against *K pneumoniae*; ampicillin against lactose-fermenting Enterobacteriaceae; and clindamycin or metronidazole against *B fragilis*.

### Biological Response Modifiers and Immunomodulating Agents

Prompt therapeutic interventions that enhance the innate immune responses in a natural manner would be a critical requirement for improving survival from infection after irradiation. There are specific and non-specific immunomodulating agents that have potential therapeutic value for treating infections in individuals who are immunocompromised due to ionizing radiation. The proinflammatory cytokines are key components of the initial host response to an infection. Experimental evidence has shown that cytokines and chemokines improve survival of irradiated animals.<sup>64-68</sup> Cytokines and chemokines have specific receptors on specific types of cells with specific consequences.

Nonspecific immunomodulating agents, such as those used as adjuvants for vaccines, also improved survival in animals that were given either lethal doses of ionizing radiation or nonlethal doses of radiation followed by challenge with nonlethal doses of bacteria. The optimal nonspecific immunomodulating agent would stimulate a natural cascade of the remaining innate immune responses in the irradiated host and could facilitate an earlier recovery. Drugs that have been evaluated against bacterial infections in irradiated animals include synthetic trehalose dicorynomycolate,<sup>58,59,69</sup>  $\beta$ -1,3-glucan,<sup>70</sup> 3D-monophosphoryl lipid A,<sup>69,71</sup> and 5-androstenediol.<sup>72,73</sup> Sufficient numbers of progenitor stem cells, which support the innate immune response, may remain viable in the bone marrow, perhaps because they are in a nonvulnerable phase of the cell cycle at the time of irradiation, particularly after partial-body irradiation. Further, these progenitor cells could be stimulated and revived to respond to an invading microorganism or a foreign antigen, such as occurs during infection or following transplantation of exogenous tissue. Some nonspecific immunomodulators can cause adverse side effects, such as granulomas or liver fibrosis caused by synthetic trehalose dicorynomycolate, but such nonlethal effects might be outweighed by their benefits to reduce mortality and improve recovery.

Specific immunomodulating agents, in particular the cytokines, granulocyte colony-stimulating factor (G-CSF), interleukin-1 $\beta$ , and interleukin-11 improved survival in lethally irradiated animals.<sup>74-76</sup> However, use of cytokines or their inhibitors for treating sepsis in animal models may not yet reflect a similar effect in humans in clinical trials.<sup>30</sup> They are generally provided as recombinant molecules and must be injected daily or on alternate days, but G-CSF conjugated with methionine (filgrastim) or polyethylene glycol (pegfilgrastim) is more stable, with a longer half-life

than G-CSF, and can be injected once subcutaneously. Timing of administration relative to irradiation and bacterial challenge is consequential as well. For example, interleukin-1 $\beta$  increases survival from infec-

tion with *K pneumoniae* when given after nonlethal irradiation and several days before bacterial challenge, but also decreases survival when given during the course of infection (Elliott TB, unpublished data).

### COMBINED THERAPY: IMMUNOMODULATING AND ANTIMICROBIAL AGENTS

Combined therapy overcomes the limitations of treating infections in irradiated persons with either antimicrobial agents or immunomodulating agents alone. A nonspecific immunomodulating agent, given once within 24 hours after irradiation, stimulates a natural cascade of the remaining innate immune responses while the antimicrobial agent attacks the microorganisms that cause the spreading infection. The value of nonspecific immunomodulators for treating infections has been demonstrated in irradiated animals, as noted above. Combined therapy with a broad-spectrum antimicrobial agent improved the outcome even more

than the immunomodulator alone against a higher infecting challenge dose of bacteria.

Combining specific immunomodulators, such as chemokines and cytokines, together with antimicrobial therapy has also been investigated for efficacy. The results and conclusions of various studies are inconclusive. Further, disadvantages include multiple daily injections following irradiation, as well as consequential bleeding, increased risk of introducing bacteria, and high financial cost. Further studies are needed to evaluate combination therapies following whole-body irradiation.

### FUTURE CONSIDERATIONS

#### Drug Delivery

Timed release of antimicrobial agents (oral, subcutaneous, or topical) to maintain a concentration above the minimum inhibitory concentration could further improve therapeutic efficacy following irradiation. Also, transdermal drug delivery by microneedle array on patches is likely to offer an alternative method to subcutaneous inoculation of therapeutic drugs. The microneedle array might be contraindicated for use in the immunocompromised host because multiple skin punctures could introduce skin and environmental microorganisms.

#### Antimicrobial Vaccines and Drugs

There is a continuing need for innovative vaccines and antimicrobial agents to provide unequivocal pro-

tection against resistant infectious agents at reasonable cost. Recent advances include development of newer generations of older antimicrobial agents, dual-action synergistic antimicrobial agents, and antimicrobial peptides.

#### Immunomodulators

Dosage, timing intervals, and routes of delivery of specific cytokines may soon be sufficiently practical for application in irradiated victims. Combinations of cytokine molecules show promise in experiments in laboratory animals to reduce toxicity and improve efficacy, particularly in febrile and neutropenic individuals. The inflammatory response to sepsis is complex. A combination of agents targeted at multiple pathways offers optimal chances for a successful outcome in each patient.<sup>30</sup>

### CURRENT RECOMMENDATIONS FOR MILITARY USE OR NATIONAL DISASTERS

Based on current knowledge and practice, recommendations can be made to prevent or treat infections that occur following irradiation. These recommendations are based on drugs currently approved for human use by the FDA, including vaccines and antimicrobial agents used for treating immunocompromised or neutropenic patients or those that have been shown to be efficacious in laboratory models of infection in whole-body-irradiated animals. The FDA has not approved biological response modifiers and immunomodulators, which are currently being

studied in laboratory animals, for treating infections in humans. The 1997, 2002, and 2010 guidelines for the use of antimicrobial agents in neutropenic patients<sup>2,3,53</sup> offer the best consensus opinion for treating infections in victims of irradiation. Nevertheless, the irradiated host may present specific and unique challenges for effective therapy and improved outcome.

Infection is best prevented by prior vaccination for known endemic or epidemic infectious agents. Therapeutic vaccinations given within several weeks after irradiation are not likely to immunize because

of lymphopenia. Attenuated vaccines are contraindicated because attenuated infectious agents could cause enhanced, life-threatening infection after irradiation.

After nonlethal irradiation, therapy for infection, even in the absence of physical injuries, is best achieved by either early initiation of antimicrobial therapy for demonstrated infection by endogenous or exogenous infectious agents, including known exposure to a biological warfare agent, or selective decontamination of the intestinal tract with antimicrobial agents against endogenous microorganisms. After lethal irradiation, broad-spectrum antimicrobial therapy should be started when the absolute number of neutrophilic cells decreases below 500 cells/ $\mu\text{L}$  and the number of thrombocytes decreases below 50,000 cells/ $\mu\text{L}$  in anticipation of endogenous bacterial translocation. Neutropenia and thrombocytopenia should be monitored.

Individuals should be monitored continually for signs and symptoms of infection for at least 21 days (up to 40 days in some cases). When signs or symptoms of infection do appear, antimicrobial therapy should be promptly initiated and continued for at least 14 and up to 21 days when there is no known exposure to a specific infectious agent, although the optimal duration of therapy is not definitively established. When physical injuries, such as trauma or burns, occur in addition to irradiation, antimicrobial therapeutic agents, both topical and systemic, should be promptly initiated and continued until wounds close, which occurs more slowly than normally after irradiation. When exposure

to a known infectious agent, such as an opportunistic microorganism or a biological attack agent, occurs within 7 days after irradiation, specific, recommended antimicrobial therapy should be promptly initiated and continued for 21 days. However, specifically for *B anthracis* infections, penicillin G or ciprofloxacin should be given for 6 weeks. Casualties who develop infections should be promptly transported to a hospital to ensure optimal supportive care.

Under controlled hygienic conditions (eg, in a hospital), parenteral therapy with a carbapenem and ceftazidime, with or without vancomycin, is recommended. The site of intravenous catheterization must be kept meticulously aseptic. However, in cases of mass casualties in which resources are inadequate, quinolones are recommended. Quinolones in particular offer advantages for effective antimicrobial therapy of bacterial infections after irradiation. Quinolones can be administered either orally or parenterally. They provide a broad spectrum of antimicrobial activity, principally against facultative gram-negative bacteria, with minimal activity against strictly anaerobic bacteria in the intestinal tract, thereby preserving colonization resistance against pathogenic microorganisms.

When either nonspecific or specific immunomodulators are approved for use in humans against infections, they may offer further advantages in combination with antimicrobial agents for improving the outcome of infections after irradiation by enhancing and advancing recovery of innate immune responses.

## SUMMARY

Nuclear weapons will cause combined injuries from wounds, burns, or blunt trauma together with ionizing radiation. Severe bacterial infections will also occur from endogenous and exogenous sources. Injury severity will be much greater than from either weapon or infectious agent alone. A comprehensive therapeutic regimen will be required to effectively treat these complex injuries. This chapter reviews the current state of knowledge and experimental research about the preventive and therapeutic measures available to diminish casualty numbers and ameliorate synergistic combined insults. Nevertheless, the irradiated host may present specific challenges for effective therapy and improved outcome. Bacterial, especially polymicrobial, infections are difficult to treat effectively in those who receive whole-body ionizing radiation be-

cause the innate immune responses are diminished. In general, antimicrobial agents alone cannot be expected to assure survival greater than 40% to 60%. Parenteral therapy, which can be monitored, is recommended for hospitalized patients, but oral administration would be more expedient for mass casualties. Quinolones appear to offer the broadest therapeutic application for infections after irradiation. Use of nonspecific or specific biological response modifiers or immunomodulators could improve outcome, but they are either not approved for human use or their efficacy has not been demonstrated in irradiated humans or experimental models of infection in irradiated animals. Further studies are needed to develop more efficacious drugs, particularly nonspecific immunomodulators, cytokines, and chemokines in irradiated animals.

### Acknowledgement

We are fortunate for expert reviews of the manuscript and recommendations offered by I Brook, MD, CDR, MC, US Navy (Retired), infectious disease physician and expert on principles of antimicrobial therapy following irradiation. We are also particularly grateful for the excellent technical and scientific contributions of RA Harding, MM Moore, and GS Madonna in the experimental research studies summarized in this review.

### REFERENCES

1. Walker RI. Infectious complications of radiation injury. In: Walker RI, Cervený TJ, eds. *Medical Consequences of Nuclear Warfare*. In: Zajtchuk R, Jenkins DP, Bellamy RF, Ingram VM, eds. *Textbook of Military Medicine*. Washington, DC: Department of the Army, Office of the Surgeon General, Borden Institute; 1989:67–83.
2. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011;52(4):e56–e93.
3. Hughes WT, Armstrong D, Bodey GP, et al. 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. *Clin Infect Dis*. 1997;25(3):551–573.
4. Waselenko JK, MacVittie TJ, Blakely WF, et al. Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group. *Ann Intern Med*. 2004;140(12):1037–1051.
5. Cervený TJ, MacVittie TJ, Young RW. Acute radiation syndrome in humans. In: Walker RI, Cervený TJ, eds. *Medical Consequences of Nuclear Warfare*. In: Zajtchuk R, Jenkins DP, Bellamy RF, Ingram VM, eds. *Textbook of Military Medicine*. Washington, DC: Department of the Army, Office of the Surgeon General, Borden Institute 1989:15–36.
6. Cronkite EP. The hemorrhagic syndrome of acute ionizing radiation illness produced in goats and swine by exposure to the atomic bomb at Bikini, 1946. *Blood*. 1950;5:32–45.
7. Elliott TB, Brook I, Stiefel SM. Quantitative study of wound infection in irradiated mice. *Int J Radiat Biol*. 1990; 58:341–350.
8. Brook I, Elliott TB, Ledney GD. Infection after ionizing irradiation. In: Zak O, Sande MA, eds. *Handbook of Animal Models of Infection: Experimental Models in Antimicrobial Chemotherapy*. San Diego, CA: Academic Press; 1999: 151–161.
9. Reinhard JF. Pharmacological screening. In: Foster HL, Small JD, Fox JG, eds. *The Mouse in Biomedical Research Experimental Biology and Oncology*. Vol IV. New York, NY: Academic Press; 1982: 313–327.
10. Madden DL, Fujiwara K. Selected bacterial diseases. In: Foster HL, Small JD, Fox JG, eds. *The Mouse in Biomedical Research Experimental Biology and Oncology*. Vol IV. New York, NY: Academic Press; 1982: 257–270.
11. Andes DR, Craig WA. Pharmacodynamics of fluoroquinolones in experimental models of endocarditis. *Clin Infect Dis*. 1998;27:47–50.
12. Andes DR, Craig WA. Pharmacodynamics of the new fluoroquinolone gatifloxacin in murine thigh and lung infection models. *Antimicrob Agents Chemother*. 2002;46(6):1665–1670.
13. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis*. 1998;26:1–12.
14. Falk PG, Hooper LV, Midtvedt T, Gordon JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. *Microbiol Molec Biol Rev*. 1998;62(4):1157–1170.
15. Fritz TE, Brennan PC, Giolitto JA, Flynn RJ. Interrelations between X-irradiation and the intestinal flora of mice. In: Sullivan MF, ed. *Gastrointestinal Radiation Injury, Symposium, Richland, Washington, September 25-28, 1966*. New York, NY: Excerpta Medica; 1968: 279–291.

16. Schaedler RW, Orcutt RP. Gastrointestinal microflora. In: Foster HL, Small JD, Fox JG, eds. *The Mouse in Biomedical Research Normative Biology, Immunology, and Husbandry*. Vol III. New York, NY: Academic Press; 1983: 327–345.
17. Lee KH, Hui KP, Tan WC. Thrombocytopenia in sepsis: a predictor of mortality in the intensive care unit. *Singapore Med J*. 1993;34(3):245–246.
18. North Atlantic Treaty Organization Military Committee Joint Standardization Board. *Standardization Agreement. Commander's Guide on the Effects From Nuclear Radiation Exposure During War*. 7th ed. Brussels, Belgium: NATO Standardization Agency; 2009. STANAG 2083.
19. US Departments of the Army, Navy, and Air Force. *NATO Handbook on the Medical Aspects of NBC Defensive Operations*. Washington, DC: DA; 1996. AMedP-6(B), Army Field Manual 8-9, Chap 6. <http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA434662>. Accessed August 2, 2012.
20. The Committee for the Compilation of Materials on Damage Caused by the Atomic Bombs in Hiroshima and Nagasaki. *Hiroshima and Nagasaki: The Physical, Medical, and Social Effects of the Atomic Bombings*. New York, NY: Basic Books, Inc; 1981.
21. Bebeshko V, Belyi D, Kovalenko A, Gergel O. *Health Consequences in the Chernobyl Emergency Workers Surviving After Confirmed Acute Radiation Sickness*. Vienna, Austria: International Atomic Energy Agency; 2002. IAEA-TECDOC-1300.
22. Brook I, Tom SP, Ledney GD. Quinolone and glycopeptide therapy for infection in mouse following exposure to mixed-field neutron-gamma-photon radiation. *Int J Radiat Biol*. 1993;64(6):771–777.
23. Elliott TB, Ledney GD, Harding RA, et al. Mixed-field neutrons and  $\gamma$  photons induce different changes in ileal bacteria and correlated sepsis in mice. *Int J Radiat Biol*. 1995;68:311–320.
24. Brook I, Walker RI, MacVittie TJ. Effect of antimicrobial therapy on bowel flora and bacterial infection in irradiated mice. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1988;53:709–716.
25. Lawrence JH, Tennant R. The comparative effects of neutrons and X-rays on the whole body. *J Exp Med*. 1937;66:667–688.
26. Ledney GD, Elliott TB, Landauer MR, et al. Survival of irradiated mice treated with WR-151327, synthetic trehalose dicorynomycolate, or ofloxacin. *Adv Space Res*. 1994;14:583–586.
27. Ledney GD, Elliott TB. Combined injury: factors with potential to impact radiation dose assessments. *Health Phys*. 2010;98(2):145–152.
28. McDonnel GM, Crosby WH, Tessmer CF, et al. *Effects of Nuclear Detonations on a Large Biological Specimen (Swine), Operation Plumbbob, Project 4.1*. Sandia Base, Albuquerque, New Mexico: Defense Atomic Support Agency; 1961. WT-1428.
29. Carrier CA, Elliott TB, Ledney GD. Resident bacteria in a mixed population of rhesus macaque (*Macaca mulatta*) monkeys: a prevalence study. *J Med Primatol*. 2009;38(6):397–403.
30. Aoki N, Xing Z. Use of cytokines in infection. *Expert Opin Emerg Drugs*. 2004;9(2):223–236.
31. Zanotti S, Kumar A, Kumar A. Cytokine modulation in sepsis and septic shock. *Expert Opin Investig Drugs*. 2002;11(8):1061–1075.
32. Opal SM, Cohen J. Clinical gram-positive sepsis: does it fundamentally differ from gram-negative bacterial sepsis? *Crit Care Med*. 1999;27(8):1608–1616.
33. van der Waaij D. The last epidemic: selective decontamination in the control of mortality among radiation victims. *Scand J Infect Dis Suppl*. 1982;36:141–149.
34. Silvestri L, van Saene HKF, Milanese M, Gregori D, Gullo A. Selective decontamination of the digestive tract reduces bacterial bloodstream infection and mortality in critically ill patients. Systematic review of randomized, controlled trials. *J Hosp Infect*. 2007;65(3):187–203.

35. Silvestri L, Mannucci F, van Saene HKF. Selective decontamination of the digestive tract: a life saver. *J Hosp Infect.* 2000;45(3):185–190.
36. Brook I, Elliott TB, Ledney GD, Shoemaker MO, Knudson GB. Management of postirradiation infection: lessons learned from animal models. *Mil Med.* 2004;169(3):194–197.
37. Brook I. Use of antibiotics in the management of postirradiation wound infection and sepsis. *Radiat Res.* 1988;115(1):1–25.
38. Brook I, Ledney GD. Quinolone therapy in the management of infection after irradiation. *Crit Rev Microbiol.* 1992;18:235–246.
39. Brook I. Management of infection following intra-abdominal trauma. *Ann Emerg Med.* 1988;17(6):626–632.
40. Brook I, Ledney GD. Effect of antimicrobial therapy on the gastrointestinal bacterial flora, infection and mortality in mice exposed to different doses of irradiation. *J Antimicrob Chemother.* 1994;33:63–72.
41. Falagas ME, Gorbach SL. Clindamycin and metronidazole. *Med Clin North Am.* 1995;79(4):845–867.
42. Brook I, Elliott TB, Harding RA, et al. Susceptibility of irradiated mice to *Bacillus anthracis* Sterne by the intratracheal route of infection. *J Med Microbiol.* 2001;50:702–711.
43. Brook I, Germana A, Giraldo DE, et al. Clindamycin and quinolone therapy for *Bacillus anthracis* Sterne infection in <sup>60</sup>Co-gamma-photon-irradiated and sham-irradiated mice. *J Antimicrob Chemother.* 2005;56(6):1074–1080.
44. Boeckh M, Lode H, Deppermann KM, et al. Pharmacokinetics and serum bactericidal activities of quinolones in combination with clindamycin, metronidazole, and ornidazole. *Antimicrob Agents Chemother.* 1990;34(12):2407–2414.
45. McChesney DG, Ledney GD, Madonna GS. Trehalose dimycolate enhances survival of fission neutron-irradiated mice and *Klebsiella pneumoniae*-challenged irradiated mice. *Radiat Res.* 1990;121(1):71–75.
46. Engelhard D, Marks MI, Good RA. Infections in bone marrow transplant recipients. *J Pediatr.* 1986;108(3):335–346.
47. Ledney GD, Elliott TB, Harding RA, Jackson WE III, Inal CE, Landauer MR. WR-151327 increases resistance to *Klebsiella pneumoniae* infection in mixed-field- and  $\gamma$ -photon irradiated mice. *Int J Radiat Biol.* 2000;76:261–271.
48. Madonna GS, Ledney GD, Elliott TB, et al. Trehalose dimycolate enhances resistance to infection in neutropenic animals. *Infect Immun.* 1989;57:2495–2501.
49. Brook I, Ledney GD. Oral ofloxacin therapy of *Pseudomonas aeruginosa* sepsis in mice after irradiation. *Antimicrob Agents Chemother.* 1990;34(7):1387–1389.
50. Brook I, Ledney GD. Oral aminoglycoside and ofloxacin therapy in the prevention of gram-negative sepsis after irradiation. *J Infect Dis.* 1991;164(5):917–921.
51. Brook I, Ledney GD. Short and long courses of ofloxacin therapy of *Klebsiella pneumoniae* sepsis following irradiation. *Radiat Res.* 1992;130(1):61–64.
52. Brook I, Elliott TB, Ledney GD, Knudson GB. Management of postirradiation sepsis. *Mil Med.* 2002;167 (Suppl 1):105–106.
53. Hughes WT, Armstrong D, Bodey GP, et al. 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis.* 2002;34:730–751.
54. Rubenstein E, Zhanel GG. Forum: Anti-infective research and development—problems, challenges, and solutions. The hospital physician. *Lancet Infect Dis.* 2007;7:69–70.
55. Lortholary O, Lefort A, Tod M, Chomat A-M, Darras-Joly C, Cordonnier C. Pharmacodynamics and pharmacokinetics of antibacterial drugs in the management of febrile neutropenia. *Lancet Infect Dis.* 2008;8(10):612–620.

56. Roberts JA, Lipman J. Pharmacokinetic issues for antibiotics in the critically ill patient. *Crit Care Med.* 2009;37(3):840–851; quiz 859.
57. Mueller EW, Boucher BA. The use of extended-interval aminoglycoside dosing strategies for the treatment of moderate-to-severe infections encountered in critically ill surgical patients. *Surg Infect (Larchmt).* 2009;10(6):563–570.
58. Ledney GD, Madonna GS, Moore MM, Elliott TB, Brook I. Synthetic trehalose dicorynomycolate and antimicrobials increase survival from sepsis in mice immunocompromised by radiation and trauma. *J Med.* 1992;23:253–264.
59. Madonna GS, Ledney GD, Moore MM, Elliott TB, Brook I. Treatment of mice with sepsis following irradiation and trauma with antibiotics and synthetic trehalose dicorynomycolate (S-TDCM). *J Trauma.* 1991;31:316–325.
60. Bartlett JG. Preparations and recommended dosing regimens for antimicrobial agents. In: Bartlett JG, ed. *2005-6 Pocket Book of Infectious Disease Therapy.* 13th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005: 1–17.
61. Shalit I, Kletter Y, Halperin D, et al. Immunomodulatory effects of moxifloxacin in comparison to ciprofloxacin and G-CSF in a murine model of cyclophosphamide-induced leukopenia. *Eur J Haematol.* 2001;66:287–296.
62. Patchen ML, Brook I, Elliott TB, Jackson WE. Adverse effects of pefloxacin in irradiated C3H/HeN mice: correction with glucan therapy. *Antimicrob Agents Chemother.* 1993;37(9):1882–1889.
63. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am J Health-Syst Pharm.* 2009;66:82–98.
64. Degre M. Interferons and other cytokines in bacterial infections. *J Interferon Cytokine Res.* 1996;16(6):417–426.
65. Neta R. Modulation with cytokines of radiation injury: suggested mechanisms of action. *Environ Health Perspect.* 1997;105(Suppl 6):1463–1465.
66. Neta R, Oppenheim JJ, Schreiber RD, Chizzonite R, Ledney GD, MacVittie TJ. Role of cytokines (interleukin 1, tumor necrosis factor, and transforming growth factor beta) in natural and lipopolysaccharide-enhanced radioresistance. *J Exp Med.* 1991;173(5):1177–1182.
67. Neta R, Oppenheim JJ, Wang JM, Snapper CM, Moorman MA, Dubois CM. Synergy of IL-1 and stem cell factor in radioprotection of mice is associated with IL-1 up-regulation of mRNA and protein expression for c-kit on bone marrow cells. *J Immunol.* 1994;153(4):1536–1543.
68. Neta R, Perlstein R, Vogel SN, Ledney GD, Abrams J. Role of interleukin 6 (IL-6) in protection from lethal irradiation and in endocrine responses to IL-1 and tumor necrosis factor. *J Exp Med.* 1992;175(3):689–694.
69. Peterson VM, Adamovicz JJ, Elliott TB, et al. Gene expression of hematoregulatory cytokines is elevated endogenously following sublethal gamma-irradiation and is differentially enhanced by therapeutic administration of biological response modifiers. *J Immunol.* 1994;153:2321–2330.
70. Patchen ML. Immunomodulators and cytokines: their use in the mitigation of radiation-induced hemopoietic injury. In: Bump EA, Malaker K, eds. *Radioprotectors: Chemical, Biological, and Clinical Perspectives.* Boca Raton, Florida: CRC Press; 1998:213–236.
71. Snyder SL, Walden TL, Patchen ML, MacVittie TJ, Fuchs P. Radioprotective properties of detoxified lipid A from *Salmonella minnesota* R595. *Radiat Res.* 1986;107(1):107–114.
72. Whitnall MH, Elliott TB, Landauer MR, et al. *In vivo* protection against gamma-irradiation with 5-androstenediol. *Exp Biol Med (Maywood).* 2001;226(7):625–627.
73. Whitnall MH, Elliott TB, Landauer MR, et al. Protection against gamma-irradiation with 5-androstenediol. *Mil Med.* 2002;167(2 Suppl):64–65.

74. MacVittie TJ, Monroy RL, Patchen ML, Souza LM. Therapeutic use of recombinant human G-CSF (rhG-CSF) in a canine model of sublethal and lethal whole-body irradiation. *Int J Radiat Biol.* 1990;57(4):723–736.
75. Neta R, Oppenheim JJ. Cytokines in therapy of radiation injury. *Blood.* 1988;72(3):1093–1095.
76. Redlich CA, Gao X, Rockwell S, Kelley M, Elias JA. IL-11 enhances survival and decreases TNF production after radiation-induced thoracic injury. *J Immunol.* 1996;157(4):1705–1710.