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MICROBIAL PATHOGENICITY

Susceptibility of irradiated mice to *Bacillus anthracis* Sterne by the intratracheal route of infection

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The susceptibility of sublethally irradiated mice to pulmonary infection with *Bacillus anthracis* was investigated in a mouse model. Female B6D2F1/J mice were challenged intratracheally with 4.3×10^6, 3.7×10^7 and 4.4×10^8 cfu of *B. anthracis* Sterne spores 4 days after ^{60}Co γ-radiation at a dose of 0, 1, 2, 3, 4, 5, 6 or 7 Gy. Bacterial cultures were obtained from lung, spleen homogenates and heart blood. A biphasic mode of mortality was observed, with a constant response of up to 3 or 4 Gy (up to 18% mortality), after which a sharp increase in mortality occurred (up to 100%). When irradiation was delayed beyond 15 days after inoculation, the susceptibility to *B. anthracis* infection and subsequent mortality disappeared. *B. anthracis* was recovered from the organs and blood of up to 89% of the animals. However, organisms of enteric origin were also isolated mixed with *B. anthracis* from up to 36% of the animals exposed to 3, 5 or 7 Gy. Inoculation of *B. anthracis* Δ-Sterne-1 that lacks lethal toxin and oedema toxin also induced infection with *B. anthracis*, but not translocation of enteric micro-organisms. The synergic adverse effect of exposure to γ-radiation followed by intratracheal challenge with *B. anthracis* was observed above 4 Gy. The lethal toxin of *B. anthracis* may enhance the emergence of polymicrobial infection with *B. anthracis* and enteric micro-organisms.

Introduction

Exposure to ionising radiation increases the host's risk of acquiring endogenous and exogenous infection. One potential source of exogenous infection is *Bacillus anthracis* spores. Infections with this organism are endemic in some parts of the world [1, 2] and it is a known potential biological warfare agent [2]. Therefore, the occurrence of accidental or war-related exposure to ionising radiation in conjunction with acquisition of *B. anthracis* infection is a potential hazard. This study investigated the susceptibility of the irradiated host to pulmonary *B. anthracis* infection with a mouse model and the veterinary vaccine strain, *B. anthracis* Sterne. This strain possesses plasmid pXO1 that encodes lethal toxin (LT) and oedema toxin (ET), but lacks the pXO2 plasmid that encodes the poly-D-glutamic acid anti-phagocytic capsule. The pulmonary route of acquisition of infection is the expected route of human exposure to *B. anthracis* as a biological warfare agent and is the type of *B. anthracis* infection that is most difficult to treat.

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Materials and methods

Animals

Female B6D2F1/J mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). All animals were kept in quarantine for 2 weeks before being released for use and transferred to a room with a 12-h light-dark cycle. Representative samples were examined to ensure the absence of specific bacteria and common murine diseases. Animals were maintained at a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International, in polycarbonate boxes with a filter cover (Micro-Isolator Lab Products, Maywood, NJ, USA) with hardwood chip bedding and were provided with commercial rodent ration and acidified water (pH 2.2). All experimental procedures were done in compliance with the Guide for the Care and Use of

Laboratory Animals (National Research Council, 1996) and Armed Forces Radiobiology Research Institute (AFRRI) regarding animal care and use.

Cobalt-60 γ-irradiation

Groups of four mice per box were placed in perforated Plexiglas restrainers and given sublethal doses of 1, 2, 3, 4, 5, 6 or 7 Gy at 0.4 Gy/min mid-line tissue (MLT) from bilaterally positioned ^{60}Co sources. Previous studies [3] had demonstrated that mortality occurs only at doses above 7.75 Gy. Dose determinations with Plexiglas mouse phantoms were made with a 0.5 cm^3 tissue-equivalent ionisation chamber whose calibration was traceable to the National Institute of Standards and Technology. The dose uniformity of the radiation was measured and found to be within ±3%.

Bacteria

B. anthracis Sterne spores were harvested from batch fermentations in Schaeffer's sporulation medium [4] with live spore veterinary vaccine as seed (Colorado Serum, Denver, CO, USA). Spores were stored at −70°C in glycerol/water 10%. Dilutions were made in sterile water to achieve the desired concentrations of spores. The number of inoculated organisms was verified by tube dilution and culture on solid medium.

Experimental design

Four days after irradiation, when circulating white blood cells are at their nadir and susceptibility to infection is greatest [3], each mouse was inoculated intratracheally with a determined number of spores.

Intratracheal inoculation of B. anthracis Sterne

The intratracheal (i.t.) route of instillation used was originally described by Saffiotti *et al*. [5]. Briefly, mice were anaesthetised by intraperitoneal injection of sodium pentobarbital (50 mg/kg) with a 25-gauge needle. As soon as an animal was anaesthetised, it was placed on a slanted holding board, with its back on the board and its mouth kept open by hanging the lower incisor teeth on a wire loop, while the upper incisors were retained by a rubber band.

B. anthracis Sterne spore suspension (1 ml) was drawn from a serum bottle into a 1-ml tuberculin syringe. The syringe was then fitted with a blunt 1.5″ 22-gauge pipetting needle bent at an angle of *c*. 135° at *c*. 1″ from the tip. A direct-focusing headlight, worn by the operator, provided a clear view of the pharynx after the tongue of the animal was gently pulled outward and laterally with forceps. The blunt tip of the needle was inserted under the epiglottis to uncover the larynx, and then lightly pushed between these into the tracheal lumen. The needle was inserted to the mid-trachea, a volume of 0.1 ml of spore suspension was gently injected, and the needle was withdrawn. Inspection of the pharynx was continued for a short time while the animal was kept on the board to make sure no suspension was regurgitated.

Animals were observed for survival and signs of disease for 30 days. Mice that died and mice designated for culture of tissues (after euthanasia) were processed for microbial cultures. Specimens of lung, spleen and heart blood were removed aseptically and swabbed on to Columbia sheep blood agar, xylose lysine desoxycholate or MacConkey agar, and Columbia CNA agar. The isolated micro-organisms were identified by the Vitek identification system (bio-Merieux Vitek, Hazelwood, MO, USA). *B. anthracis* Sterne was identified by colony morphology.

Survival studies

Preliminary studies demonstrated that mortality did not occur in more than a third of the mice with inoculation dosages of $<10^5$ cfu. Therefore, inoculation doses of $>10^6$ cfu (0.1 ml of 10^7 cfu/ml) were used. Three survival experiments were done in which the inoculation dose was: 4.3×10^6, 3.7×10^7 or 4.4×10^8 cfu/inoculation. Each experiment included seven or eight groups of 16 mice (for a total of 368 mice) given increasing radiation doses and one group that received no radiation.

A further experiment was conducted to find out whether a single dose of 4×10^8 spores would cause mortality over a protracted time between inoculation and irradiation. The experiment included eight groups of 12 mice (for a total of 96 mice) given 7 Gy radiation 1, 3, 7, 15, 21, 28, 50 and 63 days after inoculation of spores. One group of 12 mice was not inoculated and served as control.

Microbiological and pathological studies

Animals were killed by an overdose of intra-abdominal barbiturate anaesthesia. Sections of spleen, liver and one lobe of lung were removed under aseptic conditions for bacterial culture. The remaining lungs (in experiment 2 only) were gently perfused transtracheally with commercially buffered formalin (formaldehyde 10%). Sections of liver, spleen, large and small intestine, pancreas, femur, sternum and prominent lymph nodes were immersion-fixed in formalin.

Soft tissues were dehydrated in ethanol, paraffin embedded, sectioned at 5 μm and mounted on glass slides. Bony tissues were decalcified before dehydration. The histological sections were stained with haematoxylin and eosin and examined by light microscopy. Selected blocks were re-cut and the sections were stained by the Brown-Brenn Gram method.

Preliminary studies of the animals included in the

survival experiments in which cultures of lung, spleen and heart blood were obtained illustrated the isolation of mixed flora from *c*. 50% of the irradiated and challenged animals. The nature of the infection in irradiated and challenged animals was thus studied prospectively.

Experiment 1. A total of 144 49–58-week-old female mice was studied in the first experiment; 106 were given 7 Gy MLT ^{60}Co γ photons, and 38 were non-irradiated controls (Table 1). Five randomly selected animals were killed humanely and cultures of lung, spleen and heart blood were obtained. The irradiated and challenged group was sampled every 6 h for the first 4 days after challenge. Randomly selected mice from the control groups were sampled once a day as indicated in Table 1.

Experiment 2. A total of 150 19-week-old mice was studied in the second experiment. The mice were given 3 Gy (38 mice), 5 Gy (42 mice) or 7 Gy (42 mice) and 28 mice were not irradiated (Table 2). Cultures of organs and blood were obtained daily from five randomly selected mice in each group 1–5 days after irradiation. Tissue samples from each treatment group were placed in formalin and evaluated for pathological changes.

Experiment 3. A total of 106 mice was included in the third experiment: 53 were 15-week-old 'young' mice and 53 were 74-week-old 'old' mice (Table 3). Twenty-four (12 'young' and 12 'old') mice were not exposed to radiation but were challenged, 72 (36 'young' and 36 'old') received 7 Gy and challenge, and 10 (5 'young' and 5 'old') received only 7 Gy. This experiment was designed to determine if the age of the mice influenced the type of organisms isolated. Cultures of faecal pellets were obtained from five animals in each group. Cultures of lung, spleen and heart blood were obtained as in experiment 2.

Experiment 4. Inoculation with the Δ-Sterne-1 strain of *B. anthracis*, which does not possess the plasmid that confers the ability to produce LT and ET, was done to determine whether LT of *B. anthracis* influences the translocation of bacteria from the gastrointestinal tract of irradiated mice. Ninety-three mice, aged 21 weeks were given 7 Gy and 28 received no irradiation (Table 4). Cultures of organisms in tissues were obtained as in experiment 2.

Results

Mortality

A biphasic response pattern of mortality was observed, showing a constant response up to 4 Gy (4.3×10^6 and 3.7×10^7 cfu) or 3 Gy (4.4×10^8 cfu), after which a sharp increase in mortality occurred (Fig. 1). Mortality was between 0% and 18% in mice given up to 4 Gy and inoculated with 4.3×10^6 or 3.7×10^7 cfu. Similarly, mortality was between 24% and 28% in animals after radiation doses $\leqslant$3 Gy and inoculation of 4.4×10^8 cfu. Beyond 3 Gy the mortality rate progressed in relation to increased radiation dose in all groups, reaching 70% in mice given 4.3×10^6 cfu and 3.7×10^7cfu and 100% in those receiving 4.4×10^8 cfu. Individual survival experiments with incremental increases in doses of inoculated spores are shown in Figs. 2–4. Mortality was first observed in all experiments 48 h after inoculation and generally continued for 10 days. Mortality occurred earliest and

Table 1. Micro-organisms isolated from tissues of female B6D2F1/J mice after 7 Gy ^{60}Co γ-irradiation and intratracheal challenge with 5.2×10^8 cfu of *B. anthracis* Sterne spores (experiment 1)

Group	Days* sampled	Micro-organism(s) (number of mice positive/number of mice sampled) (% positive)		
		Lung	Heart blood	Spleen
7 Gy + *B. anthracis*	1–4	*B. anthracis* 42/47 (89%) *E. cloacae* 17/47 (36%) *Esch. coli* 2/47 (4%) *K. pneumoniae* 2/47 (4%) *Acinetobacter lowffii* 1/47 (2%)	*B. anthracis* 31/47 (66%) *E. cloacae* 14/47 (30%) *K. pneumoniae* 1/47 (2%)	*B. anthracis* 29/47 (62%) *E. cloacae* 6/47 (13%) *Esch. coli* 1/47 (2%) *K. pneumoniae* 1/47 (2%)
0 Gy + *B. anthracis*	1–4, 7	*B. anthracis* 13/25 (52%)	*B. anthracis* 11/25 (44%)	*B. anthracis* 11/25 (44%) *Streptococcus* sp. 2/25 (8%)
7 Gy	1–4	*Streptococcus* sp. 1/30 (3%)	None 0/30 (0%)	None 0/30 (0%)
0 Gy	1, 4	None 0/10 (0%)	None 0/10 (0%)	None 0/10 (0%)

*Days after intratracheal challenge with 5.2×10^8 cfu of *B. anthracis* Sterne spores. Mice were given 7 Gy or 0 Gy (sham) ^{60}Co γ-irradiation 4 days before spore challenge.

Table 2. Micro-organisms isolated from tissues of female B6D2F1/J mice after 3, 5 or 7 Gy ^{60}Co γ-irradiation and intratracheal challenge with 3.9×10^8 cfu of *B. anthracis* Sterne spores (experiment 2)

Treatment	Days* sampled	Micro-organism(s) (number of mice positive/number of mice sampled) (% positive)		
		Lung	Heart blood	Spleen
7 Gy + *B. anthracis*	3, 4	*B. anthracis* 7/8 (88%)	*B. anthracis* 4/8 (50%)	*B. anthracis* 5/8 (63%) *Ent. faecalis* 1/8 (13%)
5 Gy + *B. anthracis*	3–7	*B. anthracis* 23/25 (92%)	*B. anthracis* 13/25 (52%) *Erys. rhusiopathiae* 1/25 (4%) *Ent. faecalis* 1/25 (4%)	*B. anthracis* 19/25 (76%) *Erys. rhusiopathiae* 3/25 (12%) *Ent. faecalis* 1/25 (4%) *K. pneumoniae* 1/25 (4%)
3 Gy + *B. anthracis*	3–7	*B. anthracis* 14/23 (61%) *Erys. rhusiopathiae* 1/23 (4%)	*B. anthracis* 9/23 (39%) *Erys. rhusiopathiae* 1/23 (4%)	*B. anthracis* 12/23 (52%) *Erys. rhusiopathiae* 3/23 (13%) *Staph. auricularis* 1/23 (4%)
0 Gy + *B. anthracis*	3–7	*B. anthracis* 19/26 (73%)	*B. anthracis* 13/26 (50%)	*B. anthracis* 21/26 (81%)
7 Gy	3, 7	None 0/10 (0%)	None 0/10 (0%)	None 0/10 (0%)
5 Gy	3, 7	None 0/10 (0%)	None 0/10 (0%)	None 0/10 (0%)
3 Gy	3, 7	None 0/10 (0%)	None 0/10 (0%)	None 0/10 (0%)
0 Gy	2	None 0/10 (0%)	None 0/10 (0%)	None 0/10 (0%)

*Days after intratracheal challenge with 3.93×10^8 cfu of *B. anthracis* Sterne spores. Mice were given 0 Gy (sham), 3 Gy, 5 Gy or 7 Gy ^{60}Co γ-irradiation 4 days before spore challenge.

Table 3. Micro-organisms isolated from tissues of female B6D2F1/J mice after 7 Gy ^{60}Co γ-irradiation and intratracheal challenge with 3.9×10^8 cfu of *B. anthracis* Sterne spores (experiment 3)

Treatment	Days* sampled	Micro-organism(s) (number of mice positive/number of mice sampled) (% positive)		
		Lung	Heart blood	Spleen
7 Gy + *B. anthracis* (young mice)	1–4	*B. anthracis* 16/17 (94%)	*B. anthracis* 11/17 (65%)	*B. anthracis* 9/17 (53%)
7 Gy + *B. anthracis* (old mice)	1–4	*B. anthracis* 13/22 (59%) *E. cloacae* 2/22 (9%)† *Esch. coli* 1/22 (5%) *Ent. faecalis* 2/22 (9%)† *Staph. aureus* 1/22 (5%)	*B. anthracis* 9/22 (41%) *Esch. coli* 1/22 (5%) *Ent. faecalis* 1/22 (5%)†	*B. anthracis* 7/22 (32%) *Esch. coli* 1/22 (5%) *Ent. faecalis* 2/22 (9%)†
0 Gy + *B. anthracis* (young mice)	2, 4	*B. anthracis* 11/12 (92%)	*B. anthracis* 5/12 (42%)	*B. anthracis* 5/12 (42%)
0 Gy + *B. anthracis* (old mice)	2, 4	*B. anthracis* 4/12 (33%)	*B. anthracis* 2/12 (17%)	*B. anthracis* 3/12 (25%)
7 Gy (young mice)	3	None	None	None
7 Gy (old mice)	3	None	None	*Neisseria sicca* 1/5 (20%) *Streptococcus* sp. 1/5 (20%) *Bacillus* sp. 1/5 (20%)

*Days after intratracheal challenge with 5.2×10^8 cfu of *B. anthracis* Sterne spores. Mice were given 7 Gy or 0 Gy (sham) ^{60}Co γ-irradiation 4 days before spore challenge.
†These micro-organisms were also isolated from faecal pellets at the beginning of the study.

Table 4. Micro-organisms isolated from tissues of female B6D2F1/J mice after 7 Gy ^{60}Co γ-irradiation and intratracheal challenge with 3.6×10^8 cfu of *B. anthracis* Sterne or 1.5×10^8 Δ-Sterne-1 spores (experiment 4)

Treatment	Days* sampled	Micro-organism(s) (number of mice positive/number of mice sampled) (%)		
		Lung	Heart blood	Spleen
7 Gy + *B. anthracis* Sterne	1–3	*B. anthracis* 15/15 (100%) *Ent. faecalis* 2/15 (13%) *Erys. rhusiopathiae* 1/15 (7%) *Staph. xylosus* 1/15 (7%)	*B. anthracis* 12/15 (80%)	*B. anthracis* 10/15 (67%)
7 Gy + *B. anthracis* Δ-Sterne-1	1–3	*B. anthracis* 11/15 (73%)	*B. anthracis* 2/15 (13%)	*B. anthracis* 1/15 (7%) *Ent. faecalis* 1/15 (7%)†
0 Gy + *B. anthracis* Sterne	2, 3	*B. anthracis* 5/10 (50%)	*B. anthracis* 3/10 (30%)	*B. anthracis* 4/10 (40%)
0 Gy + *B. anthracis* Δ-Sterne-1	2, 3	*B. anthracis* 5/10 (50%)	*B. anthracis* 2/10 (20%)	*B. anthracis* 1/10 (10%)
7 Gy	3	None 0/5	None 0/5	None 0/5

*Days after *B. anthracis* Sterne or Δ-Sterne-1 challenge.
†*B. anthracis* was not recovered from this animal.

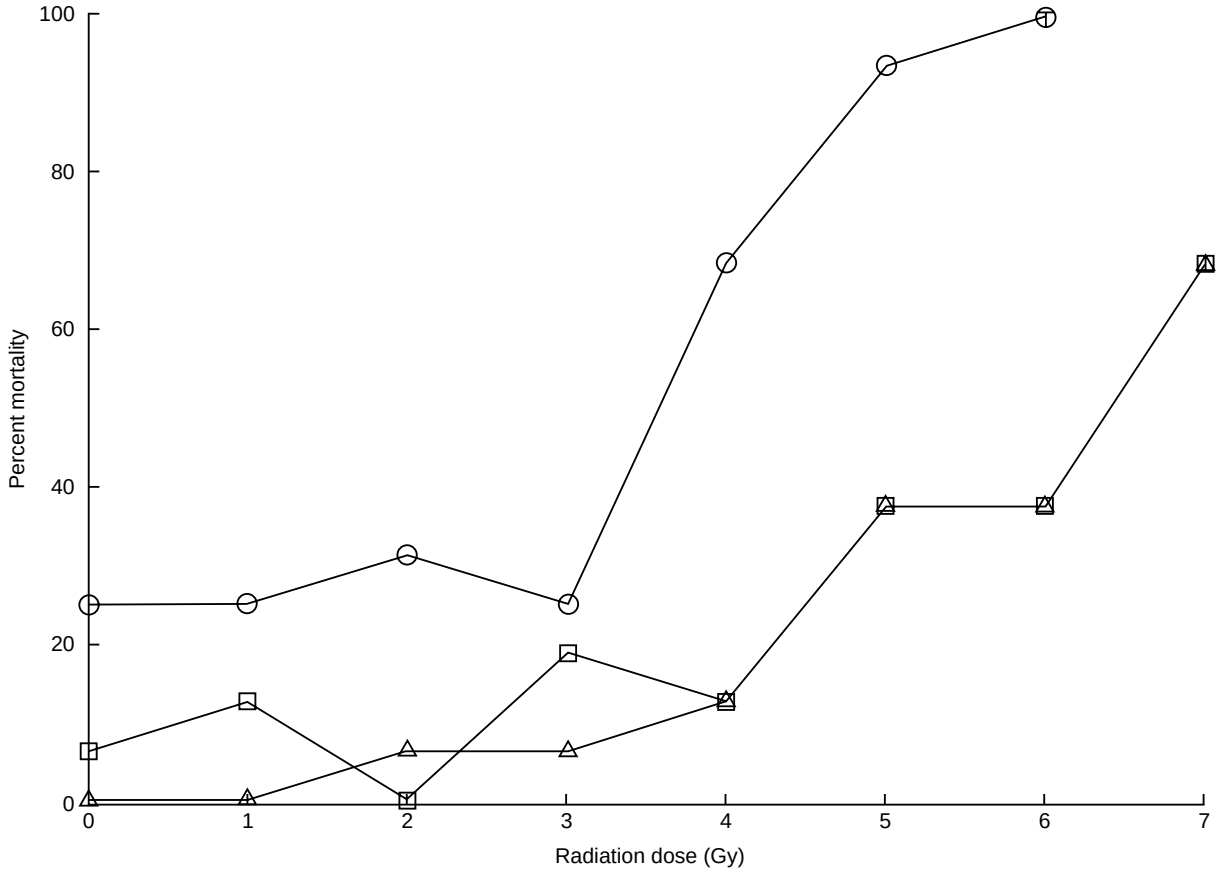

Fig. 1. Mortality of γ-irradiated B6D2F1/J mice given intratracheal challenge with *B. anthracis* Sterne spores; ○, 4.4×10^8 cfu; □, 4.3×10^6 cfu; △, 3.7×10^7 cfu.

was greatest with the high radiation doses. However, mortality after all radiation doses increased as the dose of *B. anthracis* spores was increased. The biphasic nature of the mortality was evident in all three experiments. In non-irradiated mice, mortality of 7% was noticed when 4.3×10^6 cfu of *B. anthracis* spores were inoculated (Fig. 2), 0% in those when 3.7×10^7 cfu were inoculated (Fig. 3), and *c*. 25% in those

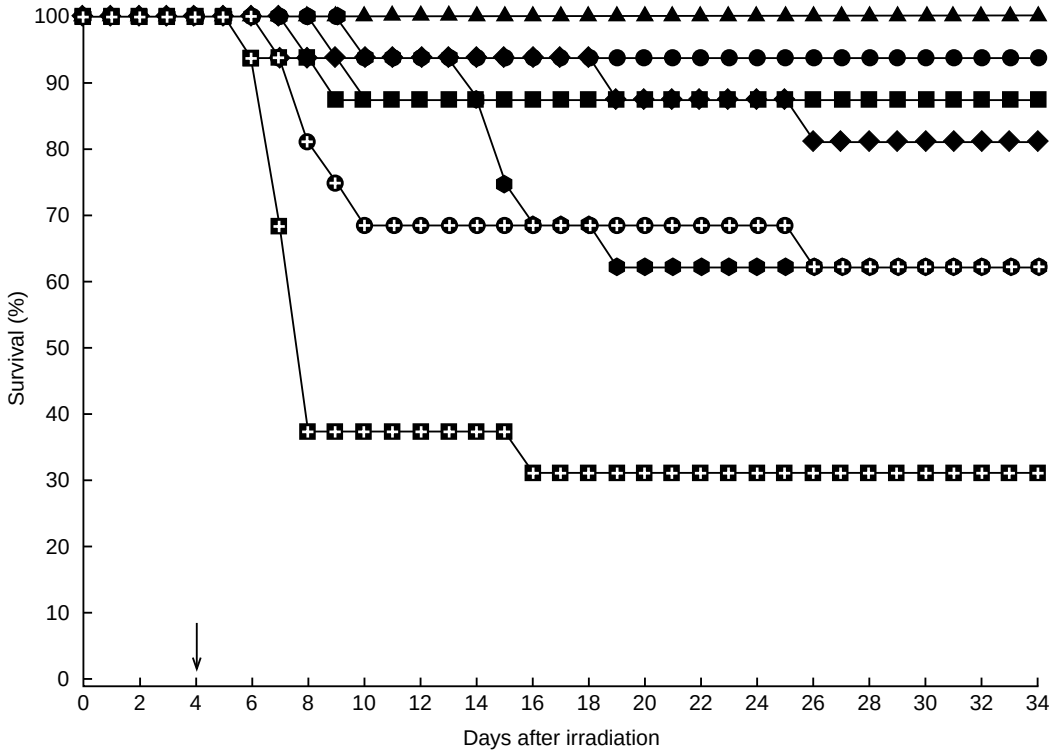

Fig. 2. Survival of B6D2F1/J mice after γ-irradiation and intratracheal challenge with 4.3×10^6 cfu of *B. anthracis* Sterne spores on day 4: ●, 0 Gy; ■, 1 Gy; ▲, 2 Gy; ◆, 3 Gy; ▼, 4 Gy; ⬣, 5 Gy; ⊕, 6 Gy; ⊞, 7 Gy.

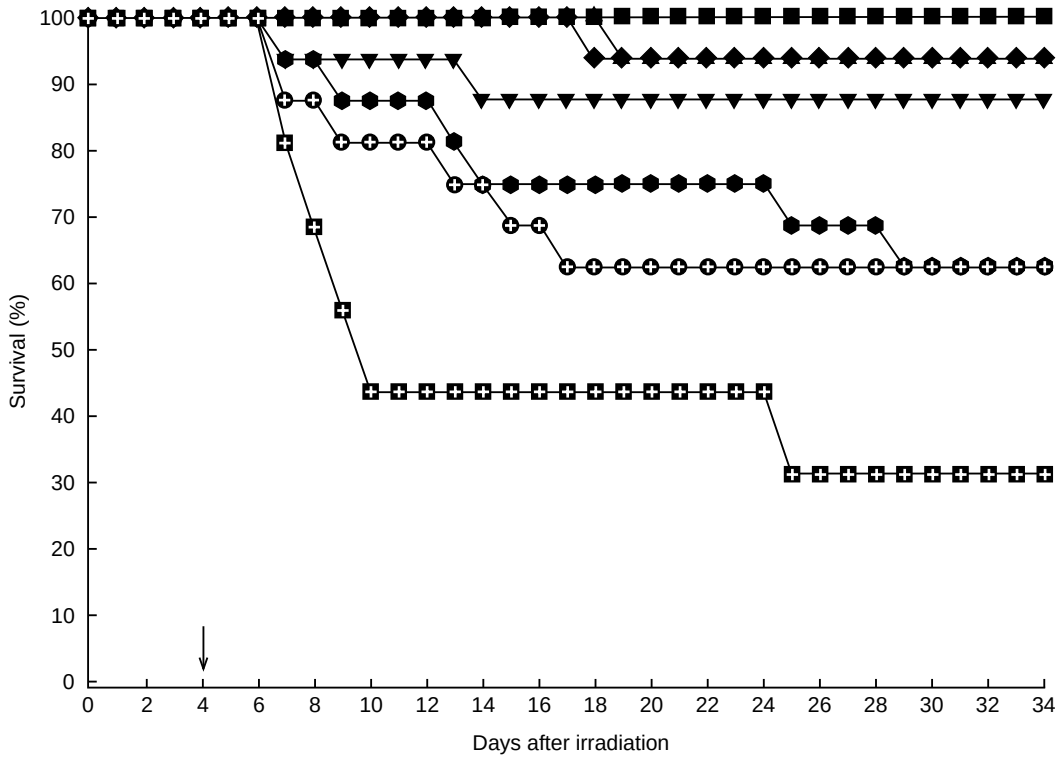

Fig. 3. Survival of B6DD2F1/J mice after γ-irradiation and intratracheal challenge with 3.7×10^7 cfu of *B. anthracis* Sterne spores on day 4. Symbols as for Fig. 2.

when 4.4×10^8 cfu were inoculated. Following 7 Gy, mortality was increased to 70% in those infected with 4.3×10^6 and 3.7×10^7 ($p < 0.001$, χ^2 test), and to 100% following 6 Gy in those challenged with 4.4×10^8 ($p < 0.001$, χ^2 test).

The highest susceptibility to inoculation with *B. anthracis* occurred when irradiation was given 1 day after bacterial challenge (100% mortality). Mortality decreased to 75% when irradiation was done on day 3 after bacterial challenge irradiation, and 50% on day 7.

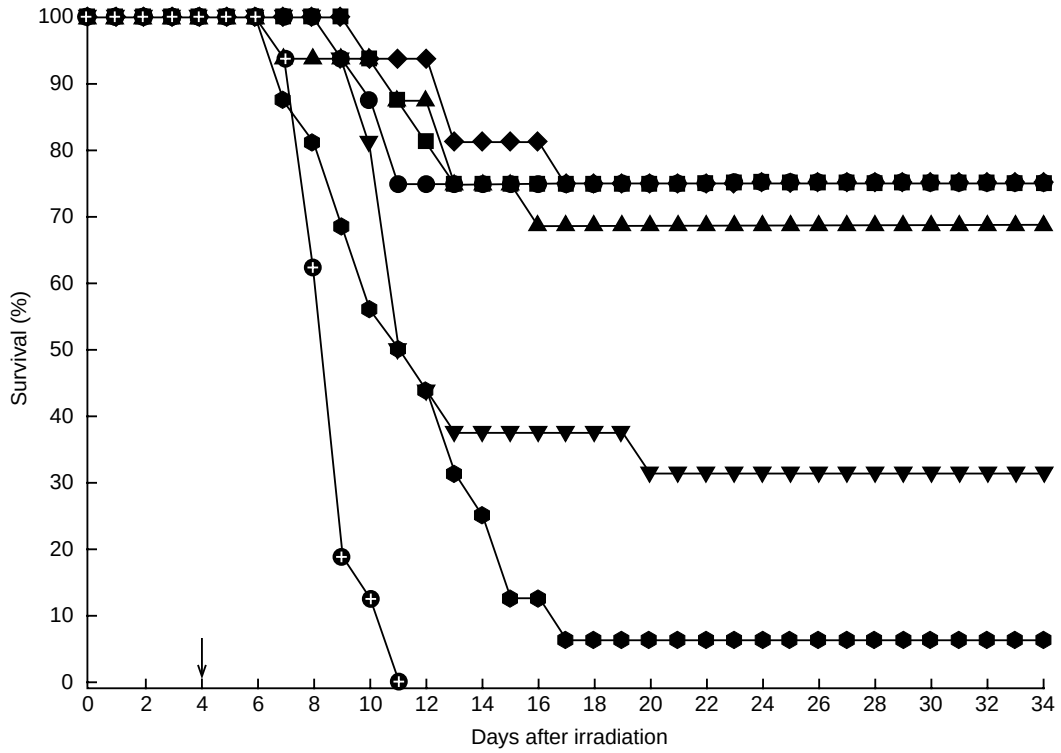

Fig. 4. Survival of B6D2F1/J mice after γ-irradiation and intratracheal challenge with 4.4×10^8 cfu of *B. anthracis* Sterne spores on day 4. Symbols as for Fig. 2.

When irradiation was given on days 15, 21, 28, 50 and 63 after inoculation of *B. antrhracis*, mortality was $<10\%$, similar to control (Fig. 5).

Pathology

The histological examination illustrated that sublethal irradiation and inoculation with *B. anthracis*, when administered together to the same mouse, caused uniformly fatal anthrax. An overwhelming bacillary bronchopneumonia with massive numbers of gram-positive bacilli was observed in the lungs (Fig. 6). The lesions were limited to individual lung lobes; other lobes from the same mouse were often unaffected.

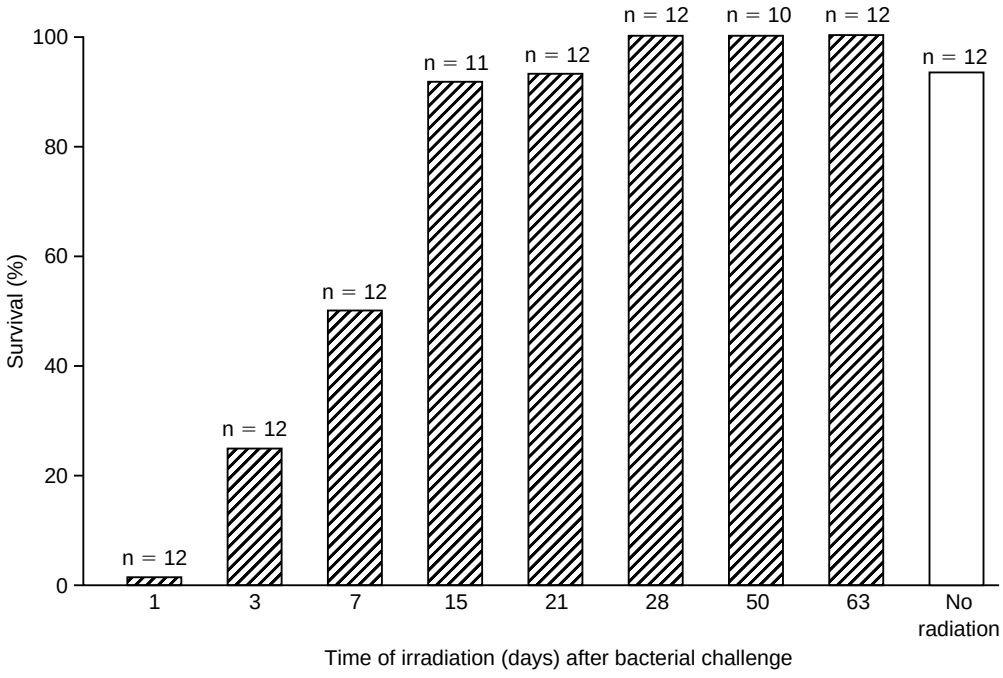

Fig. 5. Survival of mice given 7 Gy ^{60}Co γ-irradiation after intratracheal inoculation of *B. anthracis* Sterne spores (4×10^8 cfu).

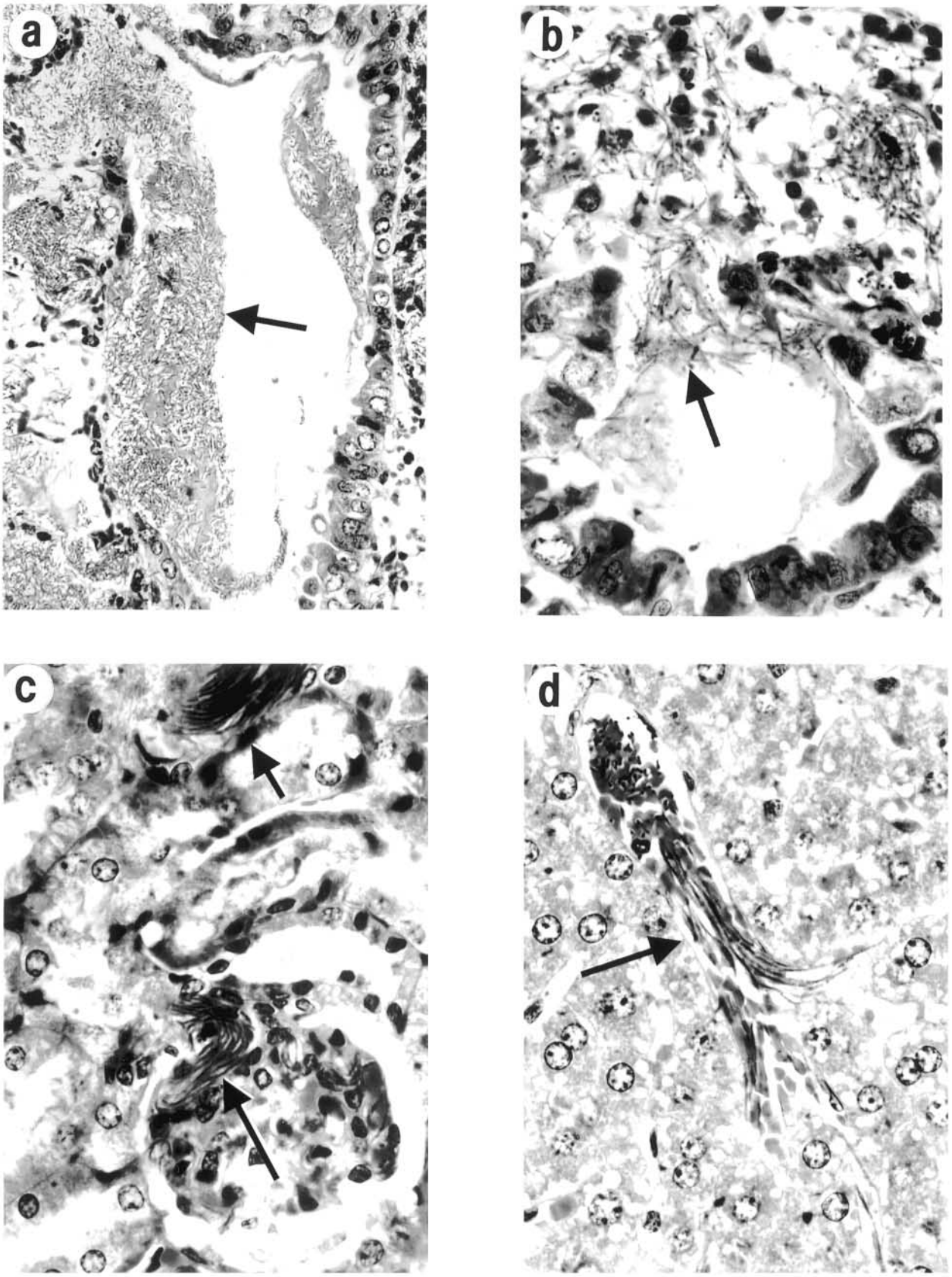

Fig. 6. Specimens of lung, liver and kidney were removed from mice killed on the third day after intratracheal challenge with *B. anthracis* Sterne spores (seventh day after irradiation). Histology sections were stained with haematoxylin and eosin. (Arrows indicates the presence of gram-positive bacilli). (**a**) Lung 400×, bacilli in bronchi and alveoli, bronchial epithelium; (**b**) lung 720×, terminal airway, bacilli in alveoli, bronchial epithelium; (**c**) kidney 720×, bacilli in afferent and efferent capillaries of glomerular tuft; (**d**) liver 720×, intravascular bacterial emboli in liver sinusoids.

Mice with pneumonia and those with lungs of normal appearance had massive numbers of intravascular micro-organisms. The bacilli were also seen in multiple organs (Fig. 6), but consistently in the capillaries of the renal pelvis.

Microbiology

In the first experiment, cultures of heart blood, lung and spleen were obtained from 47 mice given 7 Gy and challenged with *B. anthracis* Sterne spores, 25 mice challenged with spores only, 30 given 7 Gy only and 10 mice that received no radiation or challenge (Table 1). The highest recovery of *B. anthracis* was in cultures of the lung. *B. anthracis* was isolated from the lung (89%), heart blood (66%) and spleen (62%) of animals given 7 Gy and challenged. In non-irradiated mice that were only challenged, *B. anthracis* was isolated from the lung (52%), heart blood (44%) and spleen (44%). Gram-negative and non-spore-forming gram-positive micro-organisms mixed with *B. anthracis* were isolated in up to 36% of mice that were both irradiated and challenged. The predominant isolates were *Enterobacter cloacae*, *Enterococcus faecalis*, *Erysipelothrix rhusiopathiae*, *Escherichia coli* and *Klebsiella pneumoniae*. A single isolate of a *Streptococcus* species was isolated from an animal given 7 Gy. The organisms were isolated throughout all days after irradiation.

In the second experiment the animals were given 3, 5 or 7 Gy (Table 2). Cultures were obtained from 8 mice given 7 Gy and challenge, 25 given 5 Gy and challenge, 23 given 3 Gy and challenge, and 26 that were challenged but received no radiation. Ten mice each were included in the groups that were irradiated only (3, 5 or 7 Gy), or received no radiation. As noted before, *B. anthracis* was recovered from the lung, heart and spleen of all challenged animals, and other organisms were isolated from 4–13% of the animals. This time the predominant isolated species was *Erys. rhusiopathiae*.

In the third experiment, *B. anthracis* was recovered from all challenged groups, irradiated and non-irradiated, 'young' and 'old'. The highest number of isolates was in cultures of lungs. However, mixed infection was detected only in the 'old' mice given 7 Gy. Three of these isolated species were also detected in the faecal pellets at the beginning of the study (Table 3). Three single isolates were obtained from cultures of spleen from unchallenged 'old' mice.

The fourth experiment compared the recovery of micro-organisms in animals challenged with *B. anthracis* Sterne to those challenged with *B. anthracis* Δ-Sterne-1 strain. No organisms were recovered from animals that were only irradiated. *B. anthracis* was recovered from 100% and 73%, respectively, of animals challenged with either *B. anthracis* Sterne or Δ-Sterne-1. Mixed infection was present in the lungs of 4 (27%) of 15 that were irradiated and challenged with Sterne strain. However, mixed infection was *not* present in mice challenged with Δ-Sterne-1 strain (Table 4).

Discussion

This study illustrates the synergic adverse effects of exposure to ionising radiation and infection by *B. anthracis* inoculated by the intratracheal route. The animals were given sublethal doses of radiation and dosages of *B. anthracis* Sterne that induced <25% mortality in non-irradiated mice even when given at a high dose (4.4×10^8 cfu). Yet, mortality reached *c*. 70% in irradiated animals receiving 4.3×10^6 and 3.7×10^7 cfu, and 100% in those challenged with 4.4×10^8 cfu.

When irradiation was delayed beyond 15 days the susceptibility to *B. anthracis* infection and subsequent mortality was reduced to zero. This phenomenon is probably due to the recovery of the immune system over time which assists in containing the infection. However, it warrants the need for preventing exposure to this pathogen during this period.

The potential of combined exposure to ionising radiation and anthrax is a serious hazard in countries where *B. anthracis* infection is endemic. Furthermore, the chance of such occurrence is increased if biological and nuclear weapons were to be used concurrently. The data from the present study illustrate that the synergic adverse effects of *B. anthracis* together with radiation exposure take place only beyond a threshold of 3–4 Gy in mice. This dose (4 Gy) is *c*. 50% of the radiation dose that starts to induce mortality (7.75 Gy) and is 45% of the LD50/30 dose of 9.05 Gy. These findings suggest that if such combined exposure occurs, those exposed to lower dosages of radiation may not be susceptible to the adverse synergic effects of *B. anthracis* and radiation. However, beyond a certain radiation dose, such a synergic adverse effect occurs. Synergy between anthrax and ionising radiation was previously described by Berdjis *et al*. [6], who showed that combination of the two was lethal to dogs.

The present study also demonstrated the recovery of organisms other than *B. anthracis* in the organs and blood in over a third of the mice. The predominant organisms were *E. cloacae*, *Ent. faecalis*, *Erys. rhusiopathiae*, *Esch. coli* and *K. pneumoniae*. Their isolation is most probably due to their endogenous spread from the gastrointestinal tract where they are part of the normal flora in mice [7]. These organisms are cultured from 10–90% of the gastrointestinal tract flora in mice. These findings are in concordance with previous studies where these organisms were mostly isolated from animals given lethal doses of radiation [8]. However, translocation of gastrointestinal organisms was previously observed mainly when higher

doses of radiation >7 Gy were used. Similarly, in these experiments almost no translocation was observed in animals that were only irradiated. Therefore, infection with *B. anthracis* lowers the threshold for such mixed infection and is probably due to LT and ET as well as other pXO-1-associated genes/phenotypic characteristics produced by the organism, as the non-toxin-producing strain (*Δ*-Sterne-1) did not induce this phenomenon. LT is the primary virulence factor in anthrax [9], exerting its effect through lysis of the macrophages, with release of interleukin-1, followed by systemic shock and death [10]. The recovery of mixed infection where *B. anthracis* is isolated with other pathogens is, therefore, a situation unique to the irradiated host. This may require a unique therapeutic approach to victims of *B. anthracis* infection who are also exposed to ionising radiation. The use of antimicrobial agents that are effective against not only *B. anthracis* but also against all potential pathogens, (exogenous as well as endogenous) may be required [11].

Treatment with antimicrobial agents together with prior immunisation against *B. anthracis* might modify the synergic adverse effects of anthrax infection and exposure to ionising radiation. However, further studies are warranted to explore the utility of these preventive and therapeutic approaches in the face of ionising radiation. The utility of these approaches may be more complex and unpredictable in such conditions. The effects of vaccination may not be optimal because of the immune suppression induced by irradiation, and concurrent infection due to other endogenous and exogenous pathogens may complicate the therapy of *B. anthracis* infection.

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