Relationships of X Irradiation to the Enhancement of Candida albicans Infections

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Preirradiation significantly reduced the number of Candida albicans cells required for the LD₅₀ of experimentally infected mice. The start and extent of recovery of total leukocytes of preirradiated infected mice were proportional to the dose of X rays administered. During 10 days postinfection, heterophils of infected mice preirradiated with 400 R recovered to levels above unirradiated, uninfected controls but did not exceed those of unirradiated, infected animals. On the other hand, the more radiosensitive lymphocytes were depressed greatly, and a limited recovery below normal values was obtained. The lymph to heterophil ratio of uninfected mice irradiated with 400 R recovered to normal values by 11 days postirradiation. However, decreases in the ratio of unirradiated or X-irradiated infected mice showed little recovery. During 6 to 10 days postinfection, a reduction in microhematocrit values after a dose of 400 R alone was not observed when a C. albicans infection was superimposed on X-irradiated mice. This difference was not due to changes in the red blood cell volume which was decreased by 400 R with or without infection, but was attributed to a greater decrease in plasma volume caused by a combination of X irradiation and infection. Reticulocyte counts indicated that recovery from the significant decrease in erythrocyte production caused by 400 R was retarded by a progressive infection. Elevated total serum protein of unirradiated mice at 6 days after infection was attributed to increases in α- and β-globulins. A dose of 400 R limited but did not prevent an increase in α- and β-globulins upon subsequent infection. Candida species infection or 400 R or both did not greatly affect the concentration of γ-globulins. The albumin to globulin ratio of irradiated, infected mice was intermediate between those ratios found after X irradiation or infection.

Enhancement of the pathogenicity of experimental Candida albicans infections has been obtained by depressing host responses. Various agents such as mucin, cortisone, X irradiation, and certain antibiotics have been used successfully (2, 19, 27). Various mechanisms by which antibiotics alter the host resistance to this pathogenic yeast have been reviewed (20, 21). Hasenclever and Mitchell (7) found that multiplication of Torulopsis glabrata was higher in mice treated with cortisone, alloxan monohydrate, or X irradiation. The enhancement of bacterial infections by X irradiation has been investigated (14, 15).

The purpose of this study is to show the effectiveness of X irradiation in enhancing lethal experimental C. albicans infections and to describe the effects of X irradiation on leukocytes, erythrocytes, blood volume, and serum proteins during an acute infection.

MATERIALS AND METHODS
Organism and experimental infections. C. albicans A26 was grown at 30°C on Sabouraud agar as modified by Emmons (5). Viable cell suspensions were standardized by optical density determinations at 660 μm on a Coleman Model 6A spectrophotometer and were compared with a standard curve to obtain uniform inocula. CD1 male albino mice, Caesarean-born and barrier-sustained (Charles River Mouse Farms, Wilmington, Mass.), were infected intravenously in a lateral tail vein with 0.1 ml of freshly prepared Candida species cell suspension.

Values for LD₅₀ were determined by the method of Reed and Muench (18). Groups of 10 mice were separately infected with 10⁻¹, 10⁻², and 10⁻³ dilutions of 7 × 10⁶ viable C. albicans cells for virulence titration.

X-irradiation techniques. A 250-kv Westinghouse X-Ray Unit at 15 ma with a 0.25-mm copper and 1-mm aluminum filter was the X-irradiation source. The dose rate, determined by a Victoreen dosimeter, was 40 to 45 R/min. There was an average variation of 4 R from the center to the outer perimeter of the X-irradiation field. Mice were placed 40 cm from the source in a sectioned plastic-screened chamber 35.6 by 5 cm. The movement of the mice in the chamber was restricted by filling each section to capacity. The chamber was then attached to a turntable which rotated the mice 1 rev/min to insure a uniform exposure of X irradiation.
Hematological techniques. A model A Coulter Counter was used to count white blood cells. Counting suspensions of whole blood in saline were cleared of red blood cells by the lysing action of 1% saponin. Mice were bled from the orbital sinus by use of heparinized capillary tubes. The total number of leukocytes was recorded as the average of duplicate counts performed on each of three mice. Counts of lymphocytes and heterophils were obtained by the use of duplicate heparinized capillary tubes of blood from each of three mice in an International Microhematocrit Centrifuge. Reticulocyte counts were carried out according to the procedures described by Frankel and Rinehart (6).

Radioactive tracer techniques and serum protein analyses. Blood volume was measured by use of radiochromate-51Cr (Na261CrO4) (17, 24). The specific activity of the radioactive tracer containing 6 × 10−4 mcg of Cr per ml was 334 mc per mg of Cr. A 10-ml pool of mouse blood was labeled in vitro by adding 10 μc of 51Cr per ml to red blood cells suspended in saline and then incubating the radioactive suspension at 37°C for 2 hr in a Dubnoff shaker incubator. The labeled cells were washed three times with saline by centrifugation at 1,000 × g and then resuspended in the original plasma. Labeled red blood cells were prepared each day determinations were made. Blood volumes for each experimental parameter were expressed as absolute values by use of the average of five mice. The plasma volume was calculated as the difference between the total blood volume and the red blood cell volume with a hematocrit value corrected for the uneven distribution of red blood cells in total body blood and venous blood. At prescribed intervals, mice were weighed and injected intravenously with 0.2 ml of 51Cr-labeled blood. After a 5-min period for thorough mixing of blood, each mouse was anesthetized lightly with ether and bled by opening the chest cavity and collecting blood with a Pasteur pipette from a pool formed by severing the brachial vessels. In addition to use of blood samples for microhematocrit determination and reticulocyte count, 0.5 ml of blood was added to tubes containing 1 ml of Alsever’s solution for counting by a Packard Auto-Gamma Spectrometer model 410A. At 5% counting efficiency, approximately 30,000 counts per min was obtained from a 0.2-ml sample of freshly labeled blood counted each day prior to injection.

Serum protein fractions were analyzed with a Spinco model R electrophoresis system by use of the Beckman Procedure A staining method and the Analytrol scanner. Blood samples were taken at 6, 8, and 10 days postinfection. At each sampling, nine animals from each experimental group were randomly divided into three sets. The blood from each set was pooled and separated by centrifugation to obtain serum. The three serum samples from each set were then analyzed separately by paper electrophoresis, and the average values for the group were obtained. Total serum protein was determined by the method of Lowry (13).

RESULTS

A dose of 100 R of X ray 24 hr preinfection did not greatly affect the number of cells of Candida species required for the 7-day LD50 (Fig. 1). However, 200, 300, 400, and 500 R were very effective in decreasing the number of cells for the 50% mortality end point.

Mice X irradiated with 100 or 200 R and infected with 1 × 106 C. albicans A26 cells exhibited increased leukocyte levels above normal counts by 5 days after X irradiation (Fig. 2). Recovery was evident by day 3 postirradiation for a dose of 100 R, day 4 for 200 R, and day 5 for 400 R. Recovery of leukocytes in uninfected mice irradiated with 100, 200, and 400 R did not commence until 6 to 7 days after X irradiation.

A dramatic decrease in leukocytes was seen 24 hr after X irradiation with 400 R (Fig. 3A). When unirradiated mice were infected, the leukocyte count was significantly higher than that of
control animals by 2 days postinfection, whereas irradiated mice required 3 to 4 days postinfection for leukocyte levels above those of mice receiving 400 R without infection. The leukocyte counts of X-irradiated mice infected with $10^6$ or $10^5$ cells proceeded to increase from day 2 postinfection. Counts of the $10^6$ challenge remained below control values, whereas those of the $10^5$ challenge recovered by 8 days postinfection to the values found for unirradiated, infected mice. Mice receiving 400 R without infection showed depressed leukocyte counts that did not start to recover until 7 to 8 days postinfection.

After 24 hr of X irradiation with 400 R, an approximate 10-fold reduction in lymphocytes compared with heterophils was observed (Fig. 3B and C). As expected, infection of unirradiated mice resulted in slight changes in the lymphocytes and caused a marked increase in heterophils. An increase in lymphocytes of irradiated mice infected with $10^6$ cells was observed by 3 to 4 days postinfection, whereas those infected with $10^5$
significant increase in lymphocytes of uninfected, irradiated animals was seen by 9 to 10 days postinfection. Recovery of heterophils of irradiated mice infected with both levels of C. albicans was found by 2 days postinfection. Although levels of heterophils of uninfected, irradiated mice were below normal and showed recovery by 6 days postinfection, the infected, irradiated mice demonstrated heterophil counts well above normal counts.

Decrease of the lymph to heterophil ratio caused by 400 R was overcome, and recovery to normal ranges was observed by 11 days after X irradiation (Fig. 4). X-irradiated, infected mice demonstrated depressed lymph to heterophil ratios, showing little recovery for 10 days postinfection.

The microhematocrit values of either X-irradiated or unirradiated infected mice did not decrease during 6 to 10 days postinfection to the extent of those of X-irradiated, uninfected animals (Fig. 5). This relationship is seen for either 10^3 or 10^6 Candida cells.

Changes in blood volume were determined by use of ^45Cr during 6, 8, and 10 days postinfection to compare the effect of a Candida infection on unirradiated and X-irradiated mice (Table 1). During 6 to 10 days postinfection, body weight loss as a result of 400 R or C. albicans infection contributed to significant reductions in weight of X-irradiated, infected animals. The diminished blood volume on day 6 of mice which received 400 R only was attributed to a reduction in erythrocytes, whereas the lowered blood volume in X-irradiated, infected animals was a result of a decrease in both erythrocyte and plasma volumes. By day 8, the reduction in blood volume in X-irradiated, infected animals was mainly caused by the significant effects of 400 R on cell and plasma volumes. A decrease in blood volumes of X-irradiated, infected, and both X-irradiated and infected animals by day 10 was a consequence of diminution of both erythrocyte and plasma volumes.

The effect of acute doses of X irradiation on plasma protein of various experimental animals has been investigated (10, 11, 15, 16). Little change was seen in the total plasma protein of infected, irradiated mice during 6 to 8 days postinfection (Table 2). However, a concomitant decrease in total plasma proteins of infected unirradiated and irradiated mice was seen 8 to 10 days postinfection. An increase in total proteins caused by X irradiation alone appeared to limit the extent of the decrease in infected, irradiated mice. The decrease in albumin of infected, irradiated mice was attributed to the Candida infection. Elevated α-globulin fractions in infected, irradiated mice followed infection. The extent of increase, however, was mediated by the effects of 400 R. Elevated β-globulin fractions of irradiated and unirradiated infected mice decreased concomitantly during 8 to 10 days postinfection. Overall, neither 400 R nor 10^6 Candida cells
<table>
<thead>
<tr>
<th>Experimental</th>
<th>Avg body wt (g) ± SE</th>
<th>Total blood vol (ml) ± SE</th>
<th>Red Blood cell vol (ml) ± SE</th>
<th>Plasma vol (ml) ± SE</th>
<th>Cell/plasma</th>
<th>Hematocrit (%) ± SE</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 6 postinfection Unirradiated, uninfected control</td>
<td>26 ± 0.10</td>
<td>1.95 ± 0.09</td>
<td>0.84 ± 0.05</td>
<td>1.11 ± 0.06</td>
<td>0.76</td>
<td>43 ± 0.98</td>
<td>5.0</td>
</tr>
<tr>
<td>X-irradiated (400 R)</td>
<td>24 ± 0.63</td>
<td>1.57 ± 0.02</td>
<td>0.55 ± 0.01</td>
<td>1.02 ± 0.03</td>
<td>0.54</td>
<td>35 ± 0.91</td>
<td>1.1</td>
</tr>
<tr>
<td>Infected (10^4 cells)</td>
<td>21 ± 1.47</td>
<td>1.66 ± 0.11</td>
<td>0.68 ± 0.05</td>
<td>0.98 ± 0.07</td>
<td>0.69</td>
<td>41 ± 0.89</td>
<td>2.4</td>
</tr>
<tr>
<td>X-irradiated (400 R), infected (10^4 cells)</td>
<td>17 ± 0.58</td>
<td>1.30 ± 0.05</td>
<td>0.48 ± 0.03</td>
<td>0.82 ± 0.03</td>
<td>0.59</td>
<td>37 ± 1.66</td>
<td>1.5</td>
</tr>
</tbody>
</table>

| Day 8 postinfection Unirradiated, uninfected controls | 26 ± 0.51 | 1.84 ± 0.04 | 0.77 ± 0.02 | 1.07 ± 0.04 | 0.72 | 42 ± 1.45 | 5.0 |
| X-irradiated (400 R) | 22 ± 2.50 | 1.33 ± 0.15 | 0.51 ± 0.08 | 0.82 ± 0.08 | 0.62 | 38 ± 1.00 | 1.4 |
| Infected (10^4 cells) | 21 ± 1.28 | 1.64 ± 0.09 | 0.71 ± 0.04 | 0.93 ± 0.06 | 0.76 | 43 ± 0.74 | 2.7 |
| X-irradiated (400 R), infected (10^4 cells) | 15 ± 0.88 | 1.19 ± 0.05 | 0.49 ± 0.02 | 0.70 ± 0.03 | 0.70 | 41 ± 1.12 | 1.1 |

| Day 10 postinfection Unirradiated, uninfected control | 29 ± 0.19 | 2.05 ± 0.06 | 0.86 ± 0.04 | 1.19 ± 0.02 | 0.73 | 42 ± 0.66 | 5.0 |
| X-irradiated (400 R) | 25 ± 0.50 | 1.52 ± 0.05 | 0.58 ± 0.05 | 0.94 ± 0.00 | 0.62 | 38 ± 2.00 | 2.8 |
| Infected (10^4 cells) | 18 ± 0.91 | 1.57 ± 0.04 | 0.69 ± 0.02 | 0.88 ± 0.02 | 0.78 | 44 ± 0.51 | 1.9 |
| X-irradiated (400 R), infected (10^4 cells) | 13 ± 0.69 | 1.23 ± 0.05 | 0.50 ± 0.03 | 0.73 ± 0.04 | 0.68 | 41 ± 1.77 | 1.8 |

* Mice X-irradiated 24 hr prior to infection.
* Corrected by factor of 0.9.

Table 2. Influence of Candida albicans infection or X irradiation or both on serum proteins during 6, 8, and 10 days postinfection

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Conc (mg/ml)</th>
<th>A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein</td>
<td>Albumin</td>
</tr>
<tr>
<td></td>
<td>6 8 10 6 8 10 6 8 10 6 8 10 6 8 10</td>
<td></td>
</tr>
<tr>
<td>Unirradiated, uninfected control</td>
<td>55.0 55.0 54.0 36.3 34.3 33.4</td>
<td>8.1</td>
</tr>
<tr>
<td>400 R (24 hr preinfection)</td>
<td>52.2 50.0 54.3 35.1 33.6 32.8</td>
<td>6.2</td>
</tr>
<tr>
<td>10⁶ Candida cells</td>
<td>61.6 66.2 54.5</td>
<td>18.1 15.5 17.9</td>
</tr>
<tr>
<td>10⁷ Candida cells + 400 R</td>
<td>56.0 54.0 54.5 22.3 22.4 24.8</td>
<td>9.0</td>
</tr>
</tbody>
</table>

* Refers to number of days postinfection.

greatly affected γ-globulins; however, the decrease on day 8 postinfection in unirradiated, infected mice was also observed in irradiated, infected animals.

Although 400 R did not greatly affect the albumin to globulin (A/G) ratio, infection with Candida species significantly reduced the ratio. The A/G ratio values of X-irradiated, infected mice were intermediate between those of X-irradiated or infected mice. It appears that a dose of 400 R moderated both the decrease in albumin and the increase in α- and β-globulins when administered 24 hr prior to infection.

**Discussion**

Miller, Hammond, and Anderle (15) found that the LD₅₀ of Pseudomonas organisms in irradiated mice was significantly lower than that in unirradiated mice when challenged during the first 3 days after exposure to 400 R. However, enhancement of the susceptibility to bacterial infection caused by X irradiation was not seen 17
days postirradiation. Roth, Friedman, and Syver-ton (19) showed a significant increase in mortality of mice X irradiated with 200, 300, or 400 R 24 hr prior to \textit{C. albicans} infection, which was propor-tional to the dose of X irradiation. Furthermore, lesions in X-irradiated animals were more general-ized and developed faster than those found in unirradiated, infected mice. The present study demonstrates that, although 100 R showed little effect, a dose of 200, 300, 400, and 500 R 24 hr prior to infecting mice with \textit{C. albicans} markedly reduced the number of \textit{Candida} cells required for the 7-day LD_{50}.

The effect of acute doses of X irradiation on leukocytes has been reviewed (8). It has been reported (22, 23) that survival of X-irradiated mice infected with \textit{P. aeruginosa} followed the increase in leukocyte counts, and that granulo-cytes played a more important role than lympho-cytes in the survival of X-irradiated mice infected with bacteria.

The total leukocyte count of X-irradiated mice infected with \textit{C. albicans} is influenced by non-lethal doses of X ray as to when recovery starts and the extent leukocytes are produced. When X-irradiated mice were challenged with a 10-fold difference in inocula of \textit{C. albicans}, the extent of recovery was proportional to the challenge. Radiosensitive lymphocytes showed little re-
covery when a \textit{Candida} infection was super-imposed on mice receiving a dose of 400 R. On the other hand, the comparatively radioresistant heterophilis demonstrated a significantly greater recovery. It has been shown (1) that, although 600 R did not affect phagocytic activity of the reticuloendothelial system, it did inhibit recovery of normal phagocytic function after blockade with carbon particles and it did prevent stimula-
tion of phagocytosis by zymosan. Phagocytosis of \textit{Candida} species by polymorphonuclear cells oc-
curs in the blood, but in the peritoneal cavity these cells are not mobilized extensively, and phagocytic activity there is accomplished by large monocytes (28). It appears that susceptibility of X-irradiated mice to \textit{Candida} infection is due in part to their inability to proliferate leukocytes and other cells for such host responses as phagocytosis.

After nonlethal doses of X ray, there is a temporal relationship between circulating lympho-cytes and response of the rabbit to an antigen (25). Both are intact immediately after irradiation, but decrease rapidly thereafter, reaching minimal levels in 24 to 48 hr. The lymphocyte count and response to an antigen require 30 days or longer for complete recovery, despite the fact that lymphoid tissues and lymph nodes appear to be fully recovered much earlier. It has been suggested that lymphocytes are the site of anti-

body production (4), and that these cells are antibody carriers (3). Therefore, a reduction of the functional capacities of lymphocytes as a result of X irradiation associated with host re-
ponses could contribute to a decrease in resistance to \textit{C. albicans} infection.

Erythroblasts are highly radiosensitive cells. However, after exposure to doses of X ray in the LD_{50} range, significant reduction in erythrocyte, hemoglobin, or hematocrit values is not seen until approximately 6 days postirradiation (8). This delayed effect is caused by the accumulating loss of erythrocytes by normally aging cells without a proportionate replacement and by latent damage to capillary walls that causes diversion of erythro-cytes into tissue spaces and lymphatics.

A proportionate decrease in plasma volume with a reduction in erythrocytes accounted for the absence of lowered microhematocrit values 6 days after preirradiated mice were infected with \textit{C. albicans}. Decreases in blood volume seen in uninfected, irradiated animals by 11 days post-
irradiation agree with those reported previously (9). The rate of body weight loss of infected, irradiated mice was significantly greater than the decrease in total blood volume. Therefore, ex-
pression of blood volume data in terms of mil-
liters per 100 g of body weight, as was done for X-irradiated rats (24), would have shown mis-
leading increases instead of actual decreases in blood volume. The role of malnutrition associ-
ated with the combined effects of X irradiation and infection is difficult to assess. However, no change has been found to occur in plasma volume in rats fasting for 7 days (24).

Mice exposed to 400 R showed the same re-
cover in reticulocytes 7 to 11 days after X ir-
radiation as that reported with rabbits exposed to 800 R (8). Between 6 to 8 days postinfection, the reticulocyte counts of infected, irradiated mice showed the effects of 400 R. However, as the ef-
fects of 400 R were diminished during 8 to 10 days postinfection, the reticulocyte count was influ-
enced by the progressing \textit{Candida} infection.

An inhibitory factor for \textit{C. albicans} in blood plasma has been reported by Louira and Brayton (12). The fungicidal factor for \textit{Candida} was found primarily associated with the \(\alpha\)-globulins and to a lesser extent with \(\beta\)-globulins. Neither the albu-
min nor \(\gamma\)-globulin factor demonstrated anti-
\textit{Candida} activity. It is interesting that the elevated total plasma protein on day 6 that decreased by day 10 postinfection in infected, unirradiated mice was attributed to changes primarily in the \(\alpha\)- and \(\beta\)-globulins. If a concomitant decrease in the fungicidal plasma factor for \textit{Candida} occurred with the lower concentration of \(\alpha\)- and \(\beta\)-globulins of infected, irradiated mice as compared with
unirradiated, infected animals, then increased susceptibility to a progressive infection could ensue.

Since *Candida* infection does not cause a significant increase in γ-globulins as some infectious microorganisms do, the effect of high doses of x-irradiation on the biosynthesis of γ-globulins may not be a significant contribution to the enhancement of a *Candida* infection.

**Literature Cited**


