Exposure to γ-Irradiation Increases Phorbol Myristate Acetate–Induced H₂O₂ Production in Human Macrophages

By Elaine K. Gallin and Spencer W. Green

Cell number, protein, and phorbol myristate acetate (PMA)-induced H₂O₂ production were measured in cultured human peripheral blood monocytes for six days after exposure to varying doses of γ-radiation. Both the number of adherent cells and the protein per dish decreased with increasing radiation doses. The dose of radiation decreasing the number of adherent cells by 37% on days 4 and 6 postirradiation was 29 Gy. Four hours postirradiation there was a small decrease in PMA-induced H₂O₂ production for doses of 7.5 Gy or greater; levels returned to normal by eight hours and increased at 24 hours postirradiation. By day 4 postirradiation significant increases in PMA-induced H₂O₂ production were noted at all radiation doses (2.5 to 50 Gy). This increase was not due to a shift in the PMA dose-response curve, a change in the time course of the PMA response, or an effect of decreased cell density on the assay system. Superoxide levels were not significantly changed in cells exposed to 20 Gy. Catalase, glutathione peroxidase, and superoxide dismutase levels also were unchanged. Culturing irradiated cells with γ-interferon increased PMA-induced H₂O₂ release, which indicated that irradiated cells retained their capacity to respond to γ-interferon. These data demonstrate that irradiation affects the PMA-induced H₂O₂ production of human monocytes in a time- and dose-dependent manner. An increase in the release of reactive oxygen intermediates by the macrophage may play a role in enhancing the deleterious effects of radiation in vivo.

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MACROPHAGES, although relatively insensitive to the effects of radiation compared with lymphocytes, exhibit time-dependent changes in cell function and survival after exposure to ionizing radiation in doses of 7.5 to 20 Gy. At four days postirradiation, mouse peritoneal macrophages exhibit a decrease in Fc-mediated phagocytosis and a decrease in Ia expression. The acid phosphatase activity of mouse peritoneal macrophages exposed to 5 Gy or more is unaffected three hours postirradiation but increases by 24 hours. In human peripheral blood monocytes, exposure to 25 or 50 Gy decreased the 6-week survival of blood monocytes in culture and diminished the rate of microbial killing of the surviving cells. In this paper, we show that exposure of human peripheral blood monocytes to γ-radiation decreases the number and size of adherent cells within 48 hours postirradiation. However, the surviving cells exhibit an increase in PMA-induced H₂O₂ production that is evident 24 hours postirradiation and maintained throughout the six-day culture period.

The production of H₂O₂ by macrophages is associated with both extravascular cytotoxicity and inhibition of intracellular pathogens. Thus, the ability of macrophages to produce H₂O₂ in response to phorbol esters or particulate stimuli is associated with cell activation. However, H₂O₂ and other reactive oxygen intermediates, in addition to protecting against pathogens, can themselves produce extensive tissue damage. It is possible that tissue damage postirradiation is due in part to augmentation of the production of H₂O₂ by the tissue macrophage.

MATERIALS AND METHODS

Cells. Human monocytes were isolated from leukopaks supplied from the National Institutes of Health blood bank by using lymphocyte separation media (Litton Bionetics, Bethesda, MD). All blood donors were advised of procedures and attendant risks in accordance with institutional guidelines. The cellular band containing lymphocytes and monocytes was washed with phosphate-buffered saline without calcium and magnesium (PBS/0 Ca,Mg) and centrifuged through fetal calf serum (FCS) (Sterile Systems, Logan, UT) at 100 g for ten minutes. The cells were resuspended in PBS/0 Ca,Mg containing 10% FCS, and placed on Percoll gradients (Pharmacia Fine Chemicals, Piscataway, NJ). The gradients were centrifuged at 1,000 g for 20 minutes at 4°C; the monocyte band was isolated and washed twice with PBS/0 Ca,Mg. The monocytes were resuspended in tissue culture medium consisting of RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10 U/mL of penicillin (Difco, Detroit), 10 μg/mL streptomycin (Difco), 0.03% L-glutamine (Sigma Chemical Co, St Louis), and 10% heat-inactivated AB human serum (Bio-bee, Boston). The mononuclear cell layer obtained after Percoll isolation contained approximately 90% monocytes as assessed by esterase staining. In most experiments, cells were plated on cluster-6 tissue culture dishes at concentrations of 1 to 2 x 10⁴ cells/well. Cells were allowed to adhere for two hours and then were washed twice to remove nonadherent cells and refed with complete tissue culture medium. In some instances cells were placed in Teflon jars after isolation and were cultured for varying periods of time before plating and irradiating.

Cell number and protein. Cell number was determined on adherent populations of cells by counting the number of adherent cells in three separate 250 x fields of the culture dish with inverted phase microscopy. The total number of cells in the dish was then determined from the area of the microscope field and the surface area of the dish. For protein measurements cells were washed three times with PBS to remove serum proteins, and 1 mL of 0.2% Triton X-100 (New England Nuclear, Boston) was added to the dishes. Cell lysates were measured by using the Bradford method (Bio-Rad, Richmond, CA). Bovine serum albumin (Bio-Rad) was used as the protein standard. Radiation does not result in any measurable serum
protein sticking to the surface of the culture dishes. Protein per cell was obtained by dividing the total protein by the number of cells. All experiments were done in triplicate or greater.

**Radiation.** Cells were irradiated at varying times after isolation bilaterally by using a cobalt 60 γ-radiation source at a constant dose rate of 5 Gy/min.

**H₂O₂ measurements.** H₂O₂ release was assayed by measuring the decrease in fluorescence associated with the oxidation of scopoletin by H₂O₂. Adherent cells were washed twice with PBS/Ca, Mg and 2 mL of reaction mixture containing PBS, 10 mmol/L glucose, and 10 to 80 mmol/L scopoletin (Sigma), and 6 purpurogallin units of horseradish peroxidase/mL (Sigma) at pH 7.1 was added to each well. The stimulus, phorbol myristate acetate (PMA) (Consolidated Midland, Brewster, NY) at a concentration of 100 ng/mL (0.01% final dimethyl sulfoxide concentration), was added to some of the wells. In a few studies 1 mmol/L sodium azide was also added to the reaction mixture. The cells were incubated with the reaction mixture at 37°C for one hour, after which the solution was removed from the cells. The fluorescence of each sample was measured by exciting at 350 nm and recording at 460 nm. Reaction mixture not exposed to cells was used as the control. Scopoletin levels was adjusted so that the scopoletin concentration was not the limiting factor, ie, the fluorescence levels were never reduced by more than 60%. H₂O₂ levels were calculated by comparing the fluorescence decrease produced by the cells with the fluorescence decrease caused by known amounts of ethyl peroxide (Polysciences, Warrington, PA).

**Superoxide anion measurements.** Superoxide was assayed by measuring the superoxide dismutase (SOD)-inhibitable reduction of cytochrome c. Cells were washed twice with Krebs-Ringer phosphate-containing glucose (KRPG), and then 2 mL of reaction mixture (KRPG containing 50 μmol/L ferricytochrome c [Sigma] with or without 0.1 mg/mL of SOD) was added to each dish. PMA was added at 100 ng/mL to the dishes. Dishes were incubated at 37°C for one hour, at which time the absorbance of the supernatant at 550 nm was measured. The value of the absorbance with SOD was subtracted from the value of the absorbance without SOD to determine superoxide levels. Superoxide levels were computed by using an extinction coefficient of 21,000.

**SOD, glutathione peroxidase, and catalase measurements.** For SOD and glutathione peroxidase measurements cells were rinsed twice with PBS and exposed to 0.2% Triton X-100 for 30 minutes at room temperature. Cell lysates were assayed for SOD activity by using the method of McCord and Fridovich. This method assays the inhibition by SOD of the reduction of ferricytochrome c by superoxide. Xanthine and xanthine oxidase were used to generate the superoxide. Glutathione peroxidase was measured on cells and sonicated them on ice for 40 seconds. Cell sonicates were then assayed for their ability to break down H₂O₂ by following the destruction of H₂O₂ spectrophotometrically at 230 nm. All enzyme assays were done at room temperature.

**γ-Interferon.** In a few studies cells were cultured in the presence of recombinant human γ-interferon (γIFN) (AmGen, Thousand Oaks, CA) for varying periods of time before assaying PMA-induced H₂O₂ release.

**RESULTS**

**Effect of radiation on cell number and cell size.** Figure 1 shows the data from one representative experiment examining the effects of radiation on the number of adherent macrophages in culture on days 2, 4, and 6 postirradiation. Exposure to 2.5 Gy did not significantly decrease the cell number on any of the days assayed. However, with increasing doses of radiation, the cell number decreased significantly. The data from days 4 and 6 from this experiment and others are plotted in Fig. 2. The data are expressed as a percentage of control (nonirradiated) values. The line drawn through the points represents a least-squares fit to a single exponential function. The D₅₀ (dose of radiation decreasing the cell number by 37%) determined from this plot was 29 Gy.

Total protein per dish, shown in Table 1, also decreased in a dose- and time-dependent manner in irradiated cells. By days 4 and 6 postirradiation, protein levels decreased by 50% or more in dishes exposed to 20 and 50 Gy. The protein-per-cell values calculated from the protein and cell number data indicated that the surviving cells were smaller than control cells. That is, the protein-per-cell levels decreased by 18% to 37% on days 4 and 6 postirradiation.

**H₂O₂ production.** PMA-induced H₂O₂ release was measured in both control and irradiated cells at varying times postirradiation. Data from a representative experiment are shown in Fig 3A. As previously reported by Nakagawara et al., the levels of PMA-induced H₂O₂ release by control (nonirradiated) macrophages decreased with time in culture. With the exception of the 5-Gy, two-day data point, all radiation doses tested produced a significant increase in H₂O₂ release compared with the control. With increasing radiation doses the decrease in H₂O₂ production, which occurred in control cells with time in culture, was abrogated. Since our data demonstrated that radiation produces a decrease in the protein per cell, the increase in H₂O₂ expressed in terms of protein could result from cells with a reduced protein content producing the same amount of H₂O₂. To determine whether this was the case, the data were expressed in terms of cell number rather than milligrams of protein. The results are shown in Fig 3B. The data are qualitatively similar and show a dose- and time-dependent increase in H₂O₂ release after irradiation.

Table 2 summarizes a number of experiments measuring H₂O₂ levels at additional time points. Because of the variability in the H₂O₂ production between different groups of cells,
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**Fig 2.** Dose response of effect of radiation on the number of adherent cells in culture at four (4) and six (6) days postirradiation. Cells were irradiated one day after isolation. Each data point represents the average ± SE of three separate studies, each done in triplicate or greater. The line drawn through the data is a least-squares fit ($R^2 = .997$).

The data are expressed as a percentage of control values. At four hours postirradiation there was actually a small but significant decrease in PMA-induced H$_2$O$_2$ production for cells exposed to 20 Gy or greater. Eight hours postirradiation, H$_2$O$_2$ release in cells irradiated with 10 Gy or more was still slightly reduced. It should be noted that the H$_2$O$_2$ levels obtained at four and eight hours were probably reduced by the myeloperoxidase activity present in monocytes 24 to 48 hours after isolation. However, the data from cells cultured for 48 hours or longer (24 hours or longer postirradiation) was not influenced by myeloperoxidase activity (see the following section on sodium azide effects). At 24 hours postirradiation, exposure to 5 Gy produced only a 16% decrease in the protein per dish (Table 1) but a 45% increase in H$_2$O$_2$. This increase was even greater on day 2 postirradiation, was maintained on day 4, and increased further on day 6 postirradiation.

In the aforementioned studies, cells were exposed to 100 ng/mL PMA. To ensure that the dose of PMA was supramaximal for both control and irradiated cells, PMA dose-response curves were obtained for both control and irradiated (20 Gy) cells four days postirradiation. As shown in Fig 4, the dose-response curves for control and irradiated cells differed only in the magnitude of the H$_2$O$_2$ released.

The preceding studies were done on human peripheral blood monocytes that were irradiated 24 hours after isolation. To determine whether similar changes occur in more mature macrophages, cells were cultured in Teflon dishes for six to 14 days, plated on tissue culture dishes, washed to remove nonadherent cells, and irradiated with 20 Gy. PMA-induced H$_2$O$_2$ was measured on days 2 and 4 postirradiation. These studies indicated that mature human macrophages also exhibit an increase in PMA-induced H$_2$O$_2$ release after exposure to γ-radiation.

**H$_2$O$_2$ measurements at a constant protein content per dish.** Since the radiation doses (20 and 50 Gy) that produce the largest increase in H$_2$O$_2$ production on days 4 and 6 also decreased the cell protein content and cell number by 50% or greater, experiments were done to investigate the effects of cell density on PMA-induced H$_2$O$_2$ release. Human monocytes were plated at varying densities, and cultured for 1, 4, or 6 days; then the PMA-induced H$_2$O$_2$ release was assayed. In two out of three experiments, H$_2$O$_2$ levels increased as the protein per dish decreased below 15 μg/dish. To ensure that the increases in H$_2$O$_2$ were due solely to an effect of radiation and not simply a decrease in the protein per dish, a series of experiments were done in which monocytes were plated at varying densities before irradiation to compensate for the decrease in cell number and protein content after irradiation. The open circles in Fig 5 plot the data from cells assayed four days postirradiation. These data represent the average of three different experiments expressed as a percentage of control H$_2$O$_2$ release. It is evident that, even under conditions in which the protein per dish did not vary, radiation produced significant increases in H$_2$O$_2$ production. The line drawn through the points represents a least-squares fit to a bioexponential function. The closed circles in Fig 5 represent the data from Table 2 that were obtained from studies in which irradiated cells were plated at the same density as control cells so that the protein per dish was less than control values at the time of the measurements (Table 1). With the exception of the 20-Gy and 50-Gy points, the two sets of data are quite similar, indicating that the increase in H$_2$O$_2$ release by irradiated cells is not an artifact of decreased cell density.

To determine whether the time course of PMA-induced

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**Table 1.** Time Course of Change in Protein per Dish Postirradiation

<table>
<thead>
<tr>
<th>Time Postirradiation (h)</th>
<th>Radiation Dose (Gy)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
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<td>8</td>
<td>95 ± 5</td>
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<tr>
<td>24</td>
<td>94 ± 5</td>
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<td>48</td>
<td>86 ± 5</td>
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<tr>
<td>96</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>144</td>
<td>87 ± 4</td>
</tr>
</tbody>
</table>

Data are expressed as a percentage of control (unirradiated) values. Human mononuclear cells were irradiated 24 hours after isolation.

*Number of data points for each time point.
Fig 3. Dose response of effect of radiation on PMA-induced H2O2 release on days 2, 4, and 6 postirradiation. (A) H2O2 release (nmol/h/mg protein). (B) H2O2 release (nmol/h/10⁶ cells). Data (mean ± SE of four measurements) are from the same experiment as in Fig 1. *Groups not significantly different from control (P > .01).

Effect of sodium azide. H2O2 release by freshly isolated human monocytes that contain high levels of myeloperoxidase is increased fourfold by the addition of sodium azide (which inhibits myeloperoxidase) to the reaction mixture. Myeloperoxidase levels decrease in monocytes during the first two days in culture so that sodium azide has little effect on H2O2 production after day 2. To confirm that the H2O2 levels measured in these studies were maximal (ie, that they could not be further enhanced by myeloperoxidase inhibitors), several studies were done in the presence of 1 mmol/L sodium azide. The addition of sodium azide to cultures ten minutes previous to and during exposure to PMA increased (3.8-fold) H2O2 levels in macrophages assayed four hours after isolation but had no effect on cells assayed either two or

Table 2. Time Course of Change in H2O2 Production Postirradiation

<table>
<thead>
<tr>
<th>Time Postirradiation (h)</th>
<th>Radiation Dose (Gy)</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10</th>
<th>20</th>
<th>50</th>
<th>n*</th>
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<tr>
<td>4</td>
<td>2.5</td>
<td>90 ± 7</td>
<td>97 ± 11</td>
<td>80 ± 5</td>
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<td>8</td>
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<td>89 ± 9</td>
<td>92 ± 6</td>
<td>95 ± 10</td>
<td>82 ± 11</td>
<td>81 ± 11</td>
<td>67 ± 7</td>
<td>9</td>
</tr>
<tr>
<td>24</td>
<td>2.5</td>
<td>114 ± 6</td>
<td>145 ± 8</td>
<td>167 ± 9</td>
<td>152 ± 8</td>
<td>163 ± 12</td>
<td>153 ± 10</td>
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<td>48</td>
<td>2.5</td>
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<td>179 ± 18</td>
<td>233 ± 30</td>
<td>330 ± 70</td>
<td>326 ± 48</td>
<td>358 ± 28</td>
<td>12</td>
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<tr>
<td>96</td>
<td>2.5</td>
<td>139 ± 5</td>
<td>163 ± 6</td>
<td>185 ± 5</td>
<td>250 ± 11</td>
<td>296 ± 13</td>
<td>373 ± 20</td>
<td>12</td>
</tr>
<tr>
<td>144</td>
<td>2.5</td>
<td>181 ± 13</td>
<td>262 ± 31</td>
<td>239 ± 33</td>
<td>463 ± 62</td>
<td>810 ± 94</td>
<td>1183 ± 290</td>
<td>12</td>
</tr>
</tbody>
</table>

Data are expressed as a percentage of control (unirradiated) values. Cells were irradiated 24 hours after isolation and assayed for PMA-induced (100 ng/mL for 60 minutes) H2O2 production at indicated times.

*Number of data points for each time point.
Catalase, glutathione peroxidase, and SOD measurements. Three enzymes that play an important role in H$_2$O$_2$ metabolism are SOD, glutathione peroxidase, and catalase. To determine whether in H$_2$O$_2$ release in irradiated macrophages was due to either an increase in SOD activity or a decrease in glutathione peroxidase or catalase activity, the activity of these enzymes in cell homogenates was measured. As shown in Table 3, the activities of all three of these enzymes were identical in control and irradiated (20 Gy) cells.

Superoxide measurements. The H$_2$O$_2$ released after PMA stimulation is produced by the dismutation of superoxide formed from the activation of an NADPH oxidase. If the increase in H$_2$O$_2$ levels postirradiation were due to an increase in the oxidase activity, the levels of superoxide released by irradiated cells might be increased as well. PMA-induced superoxide release was measured on cells four days postirradiation (20 Gy). In these studies, irradiated cells were plated at a higher density than were the control cells since Johnston et al demonstrated that superoxide release in mouse peritoneal macrophages varies inversely with cell density. As reported previously in human macrophages, the levels of superoxide released in response to PMA were lower (two- to fourfold) than the corresponding H$_2$O$_2$ levels in all groups of cells. The superoxide data showed more variability than the H$_2$O$_2$ data (possibly due in part to the lower levels). In five of eight studies, irradiated cells showed an increase in superoxide production over control cells (with increases ranging from 10% to 200%). The data shown in Table 4 summarize the data of the eight studies. The average percent changes of irradiated cells compared with control cells for superoxide, H$_2$O$_2$, and protein per dish are given. It is evident that exposure to 20 Gy of γ-radiation did not produce a significant increase in superoxide release.
**DISCUSSION**

It is generally thought that macrophages are relatively radioresistant cells. However, recent studies indicate that time-dependent changes in bacterial killing, phagocytosis, production of colony-stimulating factor, and Ia expression occur after in vitro exposure to radiation. In most of these studies it was not possible to determine whether radiation was directly affecting the macrophage. In this paper, the effects of \( \gamma \)-irradiation on Percoll- and adherence-purified human peripheral blood monocytes (\( \sim \)95% esterase-positive) were assessed so as to minimize the possibility that radiation is affecting a different cell type that is then influencing the macrophage.

Doses of \( \gamma \)-radiation as low as 2.5 Gy produced significant decreases in the number of adherent cells and the protein per dish by 48 hours postirradiation. The \( D_0 \) for the number of adherent cells remaining on days 4 and 6 postirradiation was 29 Gy. These results confirm previous studies demonstrating that, in terms of the number of viable cells, macrophages are relatively radioresistant. Our data differ from those of Kwan and Norman who calculated two \( D_0 \) values, 0.55 and 6.5 Gy for human monocytes four days postirradiation. However, in their studies, a mixed monocyte-lymphocyte fraction was irradiated, the cells were cultured in plastic tubes for four days, and then the number of large cells (or cells ingesting latex beads) was taken as a measure of the number of macrophages. The discrepancy between our findings and those of Kwan and Norman may be due to some of these differences.

In contrast to the effects of radiation on cell number, these studies demonstrate that doses of \( \gamma \)-radiation as low as 2.5 Gy produce a significant increase in PMA-induced \( \text{H}_2\text{O}_2 \) release. This increase was not due to a shift in the PMA dose response because (as shown in Fig 4) both control and irradiated cells had qualitatively similar dose-response curves. No detectable \( \text{H}_2\text{O}_2 \) was released from either irradiated or control cells in the absence of PMA, which indicated that radiation had no effect on basal \( \text{H}_2\text{O}_2 \) levels. It has been shown previously that there is an inverse relationship between the level of superoxide, another reaction oxygen intermediate, and the cell density of cultured macrophages. Therefore, experiments were done to (a) determine whether cell density affects \( \text{H}_2\text{O}_2 \) production and (b) examine the effects of radiation in the absence of any change in cell protein per dish. It should be noted that in the studies where the cell protein per dish was constant, the cell density in the irradiated cells will increase slightly since the protein per cell

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**Table 4. Superoxide and \( \text{H}_2\text{O}_2 \) Production in Radiated Macrophages**

<table>
<thead>
<tr>
<th>( \text{O}_2 )</th>
<th>Protein per Dish, Control (%)*</th>
<th>( \text{H}_2\text{O}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (%)</td>
<td>137 ± 31</td>
<td>216 ± 33†</td>
</tr>
<tr>
<td>Protein per Dish</td>
<td>110 ± 6</td>
<td>93 ± 7</td>
</tr>
</tbody>
</table>

Cells irradiated with 20 Gy 24 hours after isolation and assayed four days later. Data are means ± SE from eight different experiments. *Dishes containing radiated cells were plated at a higher cell density (1.3 x control). † \( P = 0.008 \).

**Fig 7. Effects of \( \gamma \text{IFN} \) PMA-induced \( \text{H}_2\text{O}_2 \) release by control and irradiated macrophages.** Cells were irradiated one day after isolation. (A) Cells were incubated in 100 U/mL of \( \gamma \text{IFN} \) from days 0 to 4 postirradiation and assayed on day 4. Values represent the mean ± SE of four measurements.
decreased by 18% to 37% depending on the radiation dose. These studies demonstrated that, although PMA-induced H₂O₂ release was affected somewhat by cell density (when the protein per dish was less than 15 μg), radiation still produced significant increases in H₂O₂ release when the protein per dish was unchanged (Fig 6). Comparison of Figs 2 and 5 shows not only that PMA-induced H₂O₂ release is more sensitive to radiation than is cell number but also that the relationship between PMA-induced H₂O₂ release and γ-irradiation is more complex than the relationship between cell number and γ-irradiation. The curve in Fig 6, obtained from data with comparable protein values, fits a two-exponential function, thereby indicating that radiation has multiple effects on the cells or that more than one population of cells (in terms of the H₂O₂ response) exists.

To investigate the possibility that factors released from the irradiated macrophages (or possibly a contaminating cell type) were responsible for the increase in PMA-induced H₂O₂ release postirradiation, experiments were done in which control cells were exposed to supernatant from irradiated cells. These studies indicated that the effects of radiation were not due to the release of a stimulatory factor into the media since supernatants from irradiated cells had no effect on control cells. In addition, this finding supports the view that radiation is probably acting directly on the macrophage and not on a contaminating cell type.

To determine the site in the oxidative pathway responsible for the increase in the H₂O₂-releasing capacity of irradiated macrophages, studies were done to assess the release of superoxide by PMA-stimulated cells after radiation and to measure SOD, glutathione peroxidase, and catalase levels in these cells. Those experiments indicated that at four days postirradiation (20 Gy) the SOD, glutathione peroxidase, and catalase levels as well as the PMA-induced superoxide release were not significantly different from control values. Further studies will need to be done that measure the NADPH oxidative activity in radiated and control cells to elucidate the site(s) of action of radiation on the oxidative pathway.

Interestingly, γIFN, which enhances PMA-induced H₂O₂ release in macrophages, also increases H₂O₂ levels in irradiated (20 Gy) cells. Thus, irradiated cells are still responsive to γIFN. However, γIFN has a larger effect on the control cells so that the levels of H₂O₂ released by both control and irradiated cells in the presence of γIFN are identical. A possible interpretation of these results is that there is a maximum amount of H₂O₂ that can be released by the macrophage. Therefore, irradiated cells that release more H₂O₂ in the absence of γIFN produce less H₂O₂ in response to γIFN.

The production of reactive oxygen intermediates by phagocytes is associated with increased activation since it has been linked to tumor cell cytotoxicity, the killing of bacteria, and intracellular pathogens. However, chronic stimulation of macrophages can lead to tissue damage and inflammation. It is possible that the stimulatory action of radiation on the oxidative burst of macrophages is partly responsible for the tissue damage that occurs postirradiation. For example, the mutagenic effects of ionizing radiation are due in great part to the direct formation of active oxygen intermediates, and mutagenicity may be further enhanced by additional free radicals produced by phagocytic cells. Recent studies have shown that reactive oxygen intermediates produced by stimulating neutrophils with phorbol esters are mutagenic (transforming mouse fibroblasts into malignant cells).

Several studies have demonstrated an increased activation in macrophages harvested from irradiated animals. Therefore, the in vitro increases in H₂O₂ production reported in this study may have in vivo correlates. For example, Schultz et al. have shown that doses of x-irradiation of 1 to 8 Gy increase the ability of macrophages to suppress the proliferation of MBL-2 target cells. In these studies, 6 Gy increased the cytostatic ability of macrophages from three hours to seven days postirradiation. Similar doses of whole body radiation also increased the lysosomal enzyme content of macrophages.

In vivo studies, the effects of damaged lymphocytes, infection, etc, on macrophage function cannot be separated from the direct effect of radiation on the macrophage, which makes it difficult to compare in vivo studies with the in vitro radiation data presented in this paper. However, it is evident that, if an increase in H₂O₂ production occurs postirradiation, it is likely to enhance the deleterious effects of radiation.

ACKNOWLEDGMENT

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REFERENCES

γ-RADIATION ENHANCES H2O2 RELEASE IN MACROPHAGES

Exposure to gamma-irradiation increases phorbol myristate acetate-induced H2O2 production in human macrophages

EK Gallin and SW Green