

# The Effect of $\gamma$ -Irradiation on the Adherent Capacity and Iron Metabolism of Alveolar Macrophages in Mice and Rats

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The effect of external  $\gamma$ -irradiation on the survival of alveolar macrophages (AM) and on the extracellular release of <sup>59</sup>Fe from AM-ingested [<sup>59</sup>Fe]iron hydroxide colloid was investigated *in vitro* using cells from C3H mice and Wistar rats. <sup>59</sup>Fe release from mouse AM was enhanced by irradiation in a dose-dependent fashion, but irradiation up to 112 Gy did not affect the release from rat AM. Cell survival of AM, measured by direct counting of cell nuclei of adherent AM or vital staining with crystal violet, decreased dose dependently in mice and rats, but mouse AM were more radiosensitive than rat AM.

## Introduction

Alveolar macrophages (AM) are thought to be important scavengers in the lung due to their ability to phagocytize, transport, and digest inhaled particles, after which they may receive a relatively large radiation dose directly from inhaled radioactive particles (*1*). If physiological functions of AM are affected by irradiation, the movement and metabolism of radioactive particles might be altered. However, little is known about the effect of irradiation on AM (*2-5*). In the present study, the effect of  $\gamma$ -irradiation on AM from C3H mice and Wistar rats was investigated, and it was found that radiosensitivity of AM is considerably different between the species.

## Materials and Methods

### Effect of $\gamma$ -Irradiation on <sup>59</sup>Fe Release from Alveolar Macrophages

Bronchoalveolar lavage cells were collected by lung lavage with Ca-Mg-free phosphate-buffered saline (PBS) 24 hr after instillation of [<sup>59</sup>Fe]iron hydroxide colloid into the lungs of Wistar rats and C3H/He mice. The recovered cells were washed, re-

suspended in Eagle MEM and irradiated with a <sup>137</sup>Cs unit at a dose rate of 12.5 Gy per minute. Immediately after irradiation, we plated the cells in 96-well microtest plates and allowed them to adhere for 2 hr. Adherent cells (AM) were washed with PBS to remove nonadherent cells and cultured in fresh medium up to 3 days. In some experiments, a chelating agent, Cadiethylene-triamine pentaacetic acid (DTPA), and macrophage activating substances (lipopolysaccharide and interferon  $\gamma$ ) were added to the medium. At 24, 48, and 72 hr after irradiation, we determined the rate of <sup>59</sup>Fe release by measuring the activity of <sup>59</sup>Fe in the supernatant and in the adherent cells with an automatic well type gamma counter.

### Effect of $\gamma$ -Irradiation on Survival of Alveolar Macrophages

AM were prepared, irradiated, and cultured by the same methods as described above, except that AM were not loaded with <sup>59</sup>Fe-colloid. The survival of AM was determined by two different methods. In the first method, we determined the survival (more exactly, the number of adherent AM after washing with PBS at the end of culture) by counting the number of cell nuclei with a hemocytometer after the cell membranes were solubilized by adding 1% Zapoglobin-II in PBS. In the second method, we determined the survival by using the vital staining method with crystal violet, where AM adhering to the plates after washing with PBS were stained with 0.1% crystal violet, and absorbance of each sample was measured at a wavelength of 540 nm by a spectrophotometer (*6*).

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## Results and Discussion

The effect of irradiation on the release of  $^{59}\text{Fe}$  from mouse and rat AM loaded with [ $^{59}\text{Fe}$ ]iron hydroxide colloid was investigated. As shown in Table 1,  $^{59}\text{Fe}$  release from mouse AM was enhanced dose dependently, but rat AM was not affected by irradiation up to 112 Gy. We hypothesize that enhancement of  $^{59}\text{Fe}$  release from mouse AM was due to the impairment of cellular integrity because nuclear pyknosis, fragmentation, and autolysis were observed microscopically in irradiated mouse AM. Therefore, the effect of Ca-DTPA, interferon  $\gamma$ , lipopolysaccharide, and irradiation on  $^{59}\text{Fe}$  release was studied using only rat AM. The combination of Ca-DTPA, interferon  $\gamma$ , and lipopolysaccharide remarkably increased the release of  $^{59}\text{Fe}$  from AM, but  $\gamma$ -irradiation showed no effect (Table 2). Next we investigated the effect of irradiation on the survival of AM from both species. The survival of mouse AM estimated by the direct cell nuclei counting method was decreased to 68, 52, and 42% of nonirradiated controls by 28, 56, and 112 Gy of irradiation, respectively, at 24 hr after irradiation.

On the other hand, the survival of rat AM did not decrease. The estimation of the survival of AM by vital staining with crystal violet was more sensitive than that by direct cell nuclei counting. Also in this assay system, mouse AM were more radiosensitive than rat AM at every time point and at all doses examined (Fig. 1). The survival curve of AM was also different between these species; it was exponential with dose in mice, but not exponential in rats.

In conclusion, we found that AM from Wistar rats were relatively more radioresistant than AM from C3H mice with respect to survival.

**Table 1.** The effect of  $\gamma$ -irradiation on  $^{59}\text{Fe}$  release from mouse and rat alveolar macrophage-ingested [ $^{59}\text{Fe}$ ]iron hydroxide colloid.

Species	Dose, Gy			
	14	28	56	112
Mouse	3.7 $\pm$ 0.7 <sup>a</sup>	24.7 $\pm$ 6.1	37.3 $\pm$ 8.5	45.8 $\pm$ 6.3
Rat	3.5 $\pm$ 0.4	3.6 $\pm$ 0.6	3.4 $\pm$ 0.8	3.9 $\pm$ 1.2

<sup>a</sup> $^{59}\text{Fe}$  activity released to supernatant from alveolar macrophages during 48 hr after irradiation, expressed as percentage of whole  $^{59}\text{Fe}$  activity.

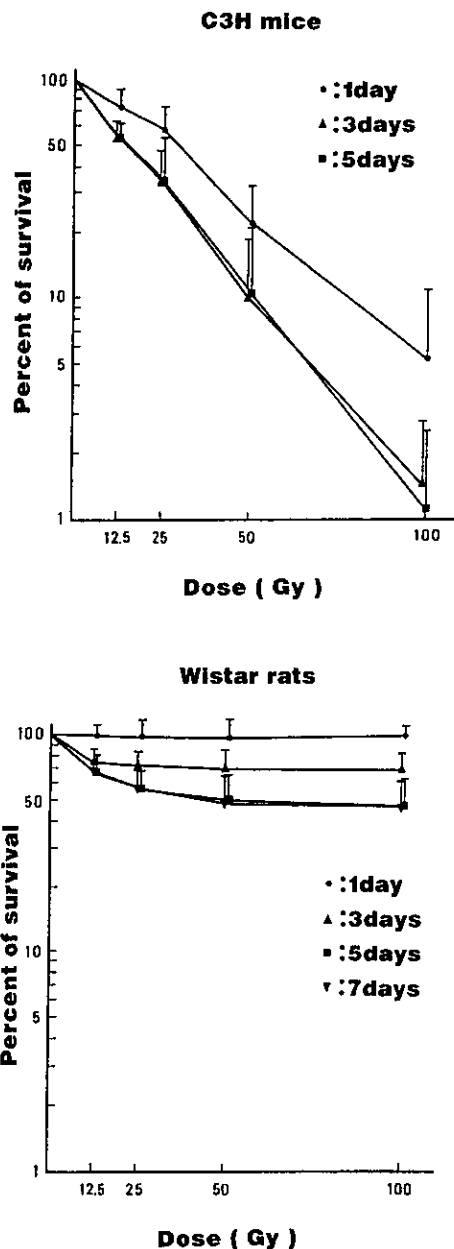
**Table 2.** Effect of Ca-DTPA, interferon  $\gamma$ , lipopolysaccharide, and  $\gamma$ -irradiation on  $^{59}\text{Fe}$  release from rat alveolar macrophages.

Ca-DTPA, mM	Irradiated dose, Gy				
	Medium	IFN- $\gamma$ <sup>a</sup>	14	28	56
Without LPS					
0	3.5 $\pm$ 0.9 <sup>b</sup>	3.6 $\pm$ 0.5	3.8 $\pm$ 0.3	3.4 $\pm$ 0.5	3.7 $\pm$ 0.2
0.33	3.4 $\pm$ 1.7	4.0 $\pm$ 0.2	4.2 $\pm$ 0.4	4.7 $\pm$ 0.3	5.0 $\pm$ 0.5
1.0	5.1 $\pm$ 1.1	6.4 $\pm$ 0.4	5.0 $\pm$ 0.4	5.9 $\pm$ 0.5	5.5 $\pm$ 0.6
3.3	6.7 $\pm$ 0.4	15.8 $\pm$ 0.4	7.2 $\pm$ 2.0	7.6 $\pm$ 0.5	7.7 $\pm$ 0.8
With 1 $\mu\text{g}/\text{mL}$ LPS					
0	5.7 $\pm$ 1.0 <sup>b</sup>	23.0 $\pm$ 2.8	6.2 $\pm$ 1.0	6.7 $\pm$ 0.4	7.0 $\pm$ 0.8
0.33	19.7 $\pm$ 2.7	72.3 $\pm$ 2.8	21.7 $\pm$ 2.2	23.2 $\pm$ 4.2	23.8 $\pm$ 2.0
1.0	35.7 $\pm$ 2.8	81.0 $\pm$ 3.7	34.8 $\pm$ 3.9	36.1 $\pm$ 3.5	36.9 $\pm$ 1.6
3.3	50.9 $\pm$ 3.8	84.8 $\pm$ 3.7	44.4 $\pm$ 2.4	43.3 $\pm$ 2.2	45.5 $\pm$ 3.8

Abbreviations: DTPA, diethylenetriamine pentaacetic acid; IFN- $\gamma$ , interferon  $\gamma$ ; LPS, lipopolysaccharide.

<sup>b</sup>Murine recombinant interferon  $\gamma$  (300 unit/mL).

<sup>c</sup> $^{59}\text{Fe}$  activity released to supernatant from rat AM during 48 hr after irradiation expressed as percentage of whole  $^{59}\text{Fe}$  activity.



**FIGURE 1.** The effect of  $\gamma$ -irradiation on the survival of alveolar macrophages (AM) from C3H mice and Wistar rats during 1-7 days after irradiation. The survival was determined by vital staining of AM with crystal violet. The survival at each dose point is expressed as percentage of nonirradiated control.

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