RELATIVE RADIATION SENSITIVITY OF CIRCULATING SMALL AND LARGE LYMPHOCYTES

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The lymphocyte has long been recognized as one of the most radiation-sensitive cells in the body. Within as little as 4 hr after receiving a radiation exposure of 100 R, the peripheral lymphocyte count is 25% of normal in 4–7-month-old rats (*1*). Lymphocytes located elsewhere in the body, such as in the lymph nodes, the thymus, the bone marrow and the spleen, have also been shown to be highly radiation sensitive although not all to the same degree (*2*). In other words, the time between irradiation and manifestation of the effect is considerably shorter in the case of peripheral lymphocytes. Of course, this difference may be due to the different methods of assessing radiation damage, or it may also be due to a difference in the immediate reaction of the cells to a radiation exposure.

Most studies that have been made concerning the reaction of the lymphatic system and lymphocytes to radiation have concentrated on the counts or observations at times of several hours to several days after exposure. Very few studies have been done on the kinetics of lymphocytes during the first 4–8 hr after irradiation except to observe that the cell count has begun to drop off. One very interesting observation along this line is that for exposures from 600–5,000 R the rate of drop in cell count is constant with time (*1*). This has been shown to be true both in the peripheral count and in bone-marrow count although in the latter case the slope or rate of drop is much slower (*1*).

In addition to the drop in peripheral count, the lymphocyte has also been shown to be directly sensitive to radiation. Within 4–6 hr after exposure to 100–400 R, pyknotic nuclei begin to appear in lymphocytes irradiated *in vitro* and incubated at 37°C (*2,3*).

Past studies have also included the observation that the relative numbers of small and large lymphocytes are changed 3–4 days after sublethal radiation exposures (*4*). The relative number of large cells increases, and this has been attributed to a greater sensitivity of small lymphocytes and to an increase in the number of large lymphocytes due to cell division activity in the recovery phase (*4*).

The investigations to be reported here were designed to take a closer look at the kinetics of the lymphocyte reaction to radiation during the first 4–8 hr postirradiation: specifically, the radiosensitivity as a function of size has been done both *in vivo* and *in vitro*. As an accompanying experiment, culturing of normal and irradiated lymphocytes was done to see if the radiation sensitivity is at all related to the mitotic potential of the peripheral cells.

MATERIALS AND METHODS

Adult female Dutch rabbits were used for all *in vivo* and *in vitro* studies. The 10,000-curie ^{60}Co source at the UCLA Laboratory of Nuclear Medicine and Radiation Biology was used for all irradiations, and exposures listed are air doses with no correction for body absorption.

All blood samples were obtained by cardiac puncture from restrained, unanesthetized rabbits. White blood cell counts and differentials were done by standard procedures using Wright's Stain and the Coulter Counter with a special diluting fluid developed by Allen and Gudaitis (*5*).

Lymphocyte separation was performed using a combination of the iron-gum arabic method of Cassen, Hitt and Hayes (*6*) and bovine fibrinogen to eliminate the need for centrifugation (*7*). The resulting cell suspension consisted of approximately half red and half white cells, and more than 99% of the leukocytes were lymphocytes.

The cell-volume distribution curves obtained were made up of red cells in channels 2 to approximately 20 and lymphocytes in channels 20 to 100 (Fig. 1). This was demonstrated by comparing size-distribution curves of whole blood with those from whole blood to which saponin had been added 1 min

Received July 10, 1967; revision accepted Feb. 20, 1968.

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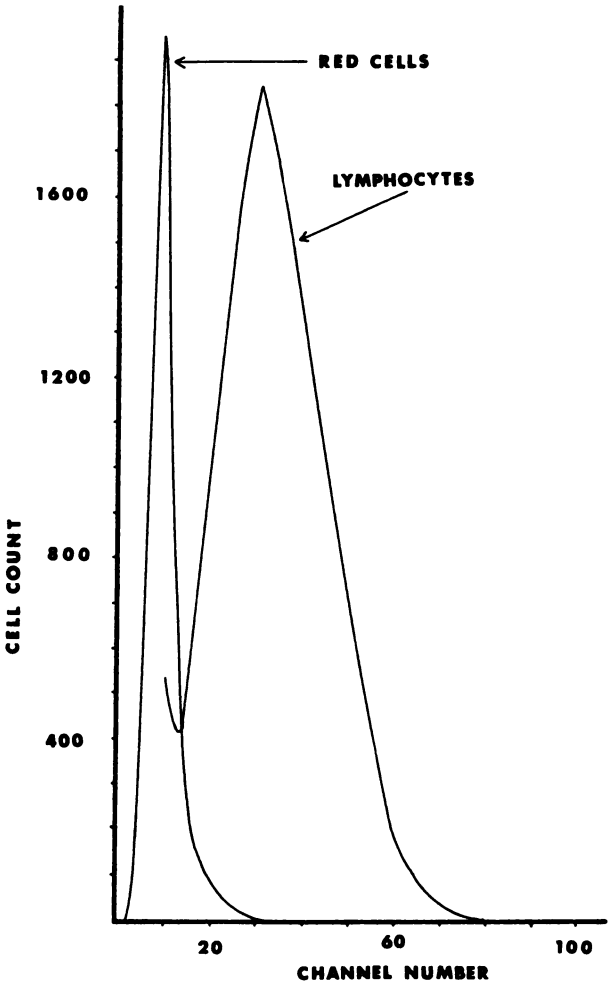

FIG. 1. Relative cell volumes of rabbit red cells and lymphocytes. Cell counts are plotted as a function of channel number.

earlier. The saponin lysed the red cells, allowing measurement of the lymphocytes alone. It is known that saponin also effects lymphocytes by shrinking them and eventually lysing them. Therefore saponin could not be used routinely to obtain pure lymphocyte size-distribution curves, but it was felt that the separation of cell volumes observed at 1 min after addition of saponin indicated that the red-cell interference in the lymphocyte channels was not prohibitive as long as cell concentrations were kept low. Cell concentrations used were well below those indicated by the Coulter Company to require any coincidence corrections.

The curves obtained were plotted as the log of cell count versus channel number or relative cell volume so that the slope would be a sensitive indicator of differences in cell-volume distributions.

Lymphocytes for the cell cultures were obtained and treated with the same procedures previously described except that sterile methods were used. Approximately 1.2×10^6 cells were cultured in Eagle's nutrient and 20% fetal calf serum. Each flask also contained 2% rehydrated Phytohemagglutinin M and 0.8 mg of Terramycin. After 3 days, 1% of the culture volume of 10^{-6} *M* colchicine was added (*8*). Eighteen hours later the cultures were terminated, fixed and prepared for microscopic counts for mitotic index (*9*).

RESULTS

The concentration of lymphocytes in the circulating blood of the rabbit is reduced to approximately 50% of normal 4 hr after irradiation with 100 R and 200 R (Table 1). In this dose range and time, the rate of fall in lymphocyte count is independent of dose received, which agrees with previous observations by Hulse (*1*).

When freshly drawn blood is irradiated *in vitro* with doses as high as 1,000 R, the fall in lymphocyte count is not nearly as rapid as that observed in *in vivo* irradiations (Table 2). As long as 8 hr after *in vitro* irradiation, the lymphocyte count, determined by standard procedures, is still 67% of normal. This is not to say that the cells have not been greatly altered, but they still would be included in cell counts by standard methods.

Whole-body irradiation of rabbits produced no changes in the relative size distribution of circulating lymphocytes 4 hr after 100 R or 200 R. A *t* test indicated that the spectral differences in 20 observations were not significant at the 0.01 level.

TABLE 1. CELL COUNTS 4 HR AFTER *IN VIVO* IRRADIATION

Dose (R)	Number of observations	Mean WBC (cells/mm³)	Lymphocyte percent of WBC	Total lymphocyte count (cells/mm³)
100	15	10,829	34.5	3,740
200	12	12,833	31.2	4,000
Controls	24	12,409	56.5	7,020

TABLE 2. CELL COUNTS AFTER 1,000 R *IN VITRO* IRRADIATION

Hours after irradiation	Number of observations	Mean WBC (cells/mm³)	Lymphocyte fraction of WBC	Total lymphocyte count (cells/mm³)
0	5	11,247	74.6	8,458
4-Irr	5	11,081	77.6	8,586
4-Con	5	11,604	77.2	8,985
8-Irr	5	9,188	62.0	5,713
8-Con	5	11,603	74.8	8,573

TABLE 3. RESULTS OF LYMPHOCYTE CELL-CULTURE EXPERIMENTS CULTURED FOR 3 DAYS

	Total cells counted	Mitotic index (mitosis/ 1,000 cells)	Standard deviation of mean
Irradiated	250,000	1.55	0.21
Control	260,000	1.30	0.17

In vitro irradiation of rabbit whole blood produced no changes in the relative lymphocyte size distribution up to 8 hr after irradiation with 1,000 R. The cells retained normal size-distribution curves.

In the cell-culture studies, the mean number of lymphocytes surviving 3 days after 300 R was 20% less than the number surviving in the control cultures as observed by cell counting. The mitotic index of cultures prepared from the blood of irradiated rabbits was not statistically different from that of cell cultures prepared from the lymphocytes of normal rabbits (Table 3). There appeared to be direct killing of circulating lymphocytes in this series of studies but killing was apparently not related to the cell-division capability, at least not in those cells which are stimulated to division by Phytohemagglutinin or during the first division postirradiation.

DISCUSSION

It has been reported and is generally accepted that the mean lymphocyte cell size, 3–14 days after irradiation with 300 R is greater than normal. This effect has been reported in thoracic duct lymphocytes and peripheral blood. It has been concluded that these shifts to larger sizes are due to direct killing of small lymphocytes and enlargement of some of the remaining cells (*4*). This then implies that the small lymphocyte is more radiation-sensitive than larger cells. When viewed during the first few hours after irradiation, however, when the change in peripheral lymphocyte count is occurring most rapidly, no such distinction in radiation sensitivity as a function of cell size is seen. The second possible explanation for the shift in cell-size distribution, that of enlargement of remaining lymphocytes, seems very likely and probably accounts for the total change in average cell size.

In the case of the immediate lymphocyte radiation sensitivity, the cells are observed to leave the circulating blood very rapidly after relatively low doses of radiation. However, since the lymphocytes in the circulating blood make up only a small percentage of the total lymphocytes in the body, this is not conclusive evidence that the reason for the rapid drop in count is due to a direct sensitivity of all lymphocytes to rapid direct killing by radiation. The question of a direct sensitivity to radiation has been studied, and it was found that 6–8 hr after exposure *in vitro* to several hundred rads, lymphocytes do begin to demonstrate morphological changes in the nucleus which eventually proceed to cell death and lysis. It must be noted, however, that the times involved in manifestation of effect are quite different when the lymphocytes are irradiated *in vivo* as compared to *in vitro* irradiation and that the effect observed is different. Other studies on *in vivo* irradiation have shown that the lymphatic system, in general, is highly radiosensitive, but here again the times involved are different. Most studies of this type list depletion of the lymphatic system in days after irradiation rather than minutes or hours.

The foregoing facts lead one then to the conclusion that the very rapid fall off in peripheral lymphocyte count after irradiation is not due to a direct killing and lysis of the cell or to a cessation of mitosis in the lymphatic system. This leaves the possibility that some organ or organs in the body recognize the circulating lymphocytes as damaged cells and remove them from circulation. Alternately as an immunologically competent cell (*10*) the lymphocyte may react to a general antigenic stimulus in essentially all the body tissues by the radiation and may therefore leave the circulation rapidly to enter into an immunological reaction. No conclusive evidence for either possibility has been observed and both remain as distinct possible answers.

With the very rapid fall in peripheral lymphocyte count after acute exposure to radiation and the implication of the lymphocyte in primary immune reactions, further investigations into the reasons for and the mechanism of this decrease in cell count should prove fruitful.

SUMMARY

Studies were made on the kinetics of the circulating lymphocyte response to radiation during the first 8 hr after irradiation and on the effect of radiation on the mitotic index of lymphocytes cultured *in vitro*. It was found that the rate of fall in peripheral lymphocyte count during the first 8 hr after 100 R and 200 R is independent of dose within that dose range and that there is no change in lymphocyte size distribution during this decrease in cell number. It was also observed that 1,000 R *in vitro* irradiation decreased the cell count at a much slower rate with still no change in size distribution.

The mitotic index of lymphocytes from irradiated animals was found to be the same as that from normal animals when cultured for 3 days in the presence of Phytohemagglutinin M. The total number

of cells surviving after 3 days was 20% less in the irradiated samples, but the fraction arrested in mitosis by colchicine was not significantly different from the controls.

ACKNOWLEDGMENT

This research was done at the UCLA Laboratory of Nuclear Medicine and Radiation Biology and was supported by U.S. Atomic Energy Commission Contract AT (04-1) GEN-12 and by the U.S. Public Health Service.

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