The Effect of Total Body Irradiation on the Labeling of Circulating Mouse Lymphocytes with Tritiated Thymidine

By D. M. Whitelaw

The small lymphocyte is considered to be uniquely radiosensitive. The dose of radiation, delivered in vivo, required to produce pyknosis in half the cells examined in the lymph nodes of young rats was 150 r.; in the circulating blood, the cells appeared more resistant, but showed the same changes. Morphologic changes have been noted in the lymphocytes of persons exposed to cyclotron radiation in small doses. In vitro studies have shown that radiation directly damages lymphocytes, causing morphologic changes and immobility with as little as 5 r. of radiation. The special sensitivity of lymphocytes was observed in experiments with extracorporeal circulation where the rapid decline in the lymphocyte counts could not have been due to irradiation of lymph nodes.

This sensitivity does not appear to depend on the size of the cell and is of the same order for lymphoblasts as for small lymphocytes. Nor does it depend on proliferative activity. Small lymphocytes rarely or never divide in the peripheral blood and do not take up tritiated thymidine either in vitro or in vivo. Although an indirect lethal effect has been postulated it appears that the destruction of lymphocytes is principally due to the direct action of radiation on the cells. It has been suggested that the mechanism is an acceleration of the normal aging process.

The present study was undertaken to determine whether young, newly formed circulating lymphocytes are more sensitive to radiation than older lymphocytes. Tritiated thymidine injected into mice labels cells which are synthesizing DNA and, if given sufficiently frequently, will label all newly formed cells. After a suitable interval two groups of circulating lymphocytes can be obtained, a younger labeled group, and an older unlabeled group. If, then, the animals are subjected to external irradiation a difference in sensitivity of younger and older lymphocytes should be detectable by a change in the ratio of labeled to unlabeled cells in the peripheral blood. If both groups are equally sensitive the ratio should remain constant despite changes in the absolute numbers of circulating lymphocytes, since each group would lose the same proportion of its members. If the younger labeled cells were more sensitive the ratio should decline and vice versa.

Methods

Three-month-old female C3Hf/Ha mice, averaging in weight 24 Gm., were used throughout. Six mice were kept in each cage and all were allowed free access to water.
Fig. 1.—The effect of 208 rads of total body irradiation on the absolute circulating lymphocyte count of mice. (Points and vertical lines represent means and standard deviations in this and subsequent graphs.)

and food (Rockland Mills mouse diet). Tritiated thymidine (obtained from the New England Nuclear Corporation) with a specific activity of 6.7 curies per millimol was diluted with saline to a concentration of 20 μC. per ml. This was injected intraperitoneally every 6 hours ± 15 minutes, in equal doses to give a total of 1 μC. per Gm. of mouse per day. Intermittent intraperitoneal injections of tritiated thymidine will succeed in labeling all dividing cells only if the interval between injections is less than the period of DNA synthesis ("S") for the type of cell under consideration. Various mammalian cells have been examined and “S” has been found to vary between 6 and 12 hours. The present technic of 6-hourly injections is therefore likely to have labeled the majority of proliferating lymph node cells.

Films for radioautography were coated with Ilford nuclear emulsion, placed in light-tight boxes with calcium chloride as a dehydrating agent and stored for 30 days in a lead castle at 4 C. They were developed in Kodak D19 developer and fixed with Kodak acid fixer. They were stained through the emulsion with Wright's stain.

The mice were irradiated at a distance of 50 cm. from a Cobalt60 gamma ray source in a plastic container revolving continuously in a plane perpendicular to the axis of the beam. The lid of the plastic container was 6 mm. thick. The dose rate was 43 r. per minute and radiation was delivered for 5 minutes in a single dose, giving an exposure of 215 r. and an absorbed dose to the mice of 208 rads. This dose was chosen because it is known to have a significant effect on lymphocytes, but is well below the minimum lethal dose for this strain of mice.

Differential counts of labeled and unlabeled lymphocytes were made by counting 100
cells from each mouse for each point in time. For each slide an estimate was made of the background grain count. A cell was considered labeled if the number of grains superimposed upon it would have occurred by chance no more than once in 20 cells. In most preparations, this was 1 gr. but in some it was 2 gr. The average labeled cell contained about 12 gr. No attempt was made to distinguish large from small lymphocytes.

RESULTS

The mean absolute lymphocyte count (fig. 1) in 60 mice was $8.3 \times 10^9$ with a standard deviation of $3.2 \times 10^9$ per cu. mm. Over a 13-day period of repeated intraperitoneal injections of tritiated thymidine there was a fall to $4.7 \pm 1.8 \times 10^9$ per cu. mm. In control animals receiving tritiated thymidine alone for a total of 21 days there was no further decrease in the total lymphocyte count. The fall that occurred before the seventh day of injection was probably, therefore, attributable to the manipulation of the mice rather than to the radiation delivered to the cells by the tritium. External irradiation with 208 rads reduced the total lymphocyte count to $0.8 \pm 0.4 \times 10^9$ per cu. mm. after 2 days. There was partial but temporary recovery reaching a peak 5 days
after irradiation and falling back before rising again. This corresponds to the phenomenon of "abortive recovery" described by others.

The percentage lymphocyte count, which began at 54 per cent, fell to 45 per cent during the initial labeling procedure (fig. 2). Following external irradiation it fell to 17 per cent on the second day, showed an "abortive recovery" phase reaching a peak in 5 days and began to recover again after 9 days. The neutrophil counts fell slightly after irradiation (fig. 3), but recovered within 3 days to normal levels. The circulating lymphocyte count was therefore more affected by radiation than the circulating neutrophil count.

In 1 series of 20 mice, blood was taken from the tail for radioautography every 3 days until the fifteenth day when the mice were exposed to external irradiation. The rate of labeling of lymphocytes was about 2.5 per cent per day. Because frequent bleeding of the mice involved the development of significant anemia, a second group of 20 mice was bled only after a period of 13 days of labeling. The rate of labeling did not differ significantly in the 2 groups (fig. 4).

In the first experiment, labeling with tritiated thymidine was discontinued on the fifteenth day at which time the dose of 208 rads of external irradiation was administered. Figure 5 shows that the decline in the percentage of circulating labeled cells was the same for irradiated as for control mice.

In the second experiment (fig. 6) tritiated thymidine was continued through-
Fig. 4.—The rate of labeling of circulating lymphocytes in mice given 6-hourly injections of tritiated thymidine.

Fig. 5.—Labeling curve of lymphocytes in mice labeled for 15 days and then exposed to 208 rads of external radiation.

out. The slope of the labeling curve was unchanged by external irradiation. This indicates that despite the fivefold reduction in the total circulating lymphocyte count, the number of new cells added per unit time bore the same relation to the number of older cells in the circulation as it had before the irradiation.
In the third experiment (fig. 7) a group of mice was subjected to a second dose of 208 rads of radiation 4 days after the first. This reduced the mean total lymphocyte count to 0.6 ± 0.2 x 10⁷ per cu. mm., but did not affect the slope of the labeling curve.

**DISCUSSION**

A single dose of 208 rads of ionizing radiation given to C3H mice caused a fivefold drop in the circulating lymphocyte count. It did not, however, alter either the ratio of labeled to unlabeled cells or the slope of the labeling curve. If the labeling procedure was continued after the radiation, the cells still became labeled at the same rate relative to the absolute count. If the labeling procedure was discontinued, the proportion of circulating labeled cells fell off at the same rate, whether or not the animals were irradiated. A second dose of radiation further depressed the total count of lymphocytes but did not immediately affect the ratio of labeled to unlabeled cells and did not change the slope of the labeling curve.

After 2 weeks of intermittent injections of tritiated thymidine, approximately one-third of the circulating lymphocytes were labeled. Those that were unlabeled must have undergone their last division more than 2 weeks previously, and were older cells. Those that were labeled must have been synthesizing DNA at some time after the commencement of injections and were therefore younger than 2 weeks old. The ratio of labeled to unlabeled cells would have been disturbed by the external irradiation if either group had been more sensitive than the other. It is important to recall that the "young" cells referred to here are nondividing cells,—mature small lymphocytes formed less than 2 weeks before but not actively proliferating. Since the ratio
of newly formed to older lymphocytes was, in fact, not altered by external irradiation, the old cells could have been no more susceptible to destruction by this dose of radiation than the young cells. It seems unlikely that any acceleration of a hypothetical aging process occurred.

It might be argued that the decline in the total lymphocyte count was due only to radiation injury to the capillaries of the mouse allowing escape of the cells into the tissues. It has indeed been shown\textsuperscript{12} that irradiation of mice with 750 r. will allow escape of red blood cells into tissues and then into the thoracic duct. In the dose used in the present experiment it seems unlikely that this was more than a minor factor.

Evidence derived from both in vivo\textsuperscript{13} and in vitro\textsuperscript{14} experiments indicates that lymphocytes are readily injured by doses of radiation comparable to those used here. Circulating lymphocytes can also be depleted by irradiation of blood in an extracorporeal circuit,\textsuperscript{9} where injury to host capillaries could not have been the responsible factor.

The external radiation must have affected the proliferative capacity of the lymph nodes as well as causing damage to the cells circulating in the peripheral blood. Had it not done so and were all the circulating cells equally susceptible, the ratio of labeled to unlabeled cells would have risen sharply as the mature circulating lymphocytes were eliminated. Had the older circulating cells been more susceptible than the younger, there would also have been an upward deviation of the slope of the labeling curve. Had the younger circulating cells been more susceptible, there would have been a fall in the ratio of labeled to unlabeled cells followed by a rise.
If the lymphopoietic tissue had been stimulated by the radiation, the labeling curve would have risen even more sharply, either at once or after an initial brief fall.

If, on the other hand, 208 rads of external radiation had caused complete cessation of lymph node activity and the circulating cells been equally susceptible, the labeling curve would slowly have risen as the older cells were eliminated. If the older circulating cells had been more susceptible to radiation than the younger, there would have been a sudden increase in the ratio followed by a phase of slower increase as the older cells were again eliminated by normal processes. If the younger cells had been more susceptible there would have been a sudden decline in the curve followed by a slower declining phase. None of these effects was observed.

The fourth and most likely possibility is that the external radiation caused immediate destruction of lymph node cells followed by active regeneration which proceeded at the same rate as before. The data presented here indicate that the output of new lymphocytes is directly related to the level of circulating lymphocytes. This relationship is the opposite of that of a feed-back mechanism but on the other hand is compatible with the concept that small lymphocytes re-enter a cycle to become proliferating cells whose progeny reach the blood stream in numbers proportional to the supply of circulating lymphocytes.

The failure to demonstrate a differential sensitivity between labeled and unlabeled or new and old lymphocytes suggests that older cells are not necessarily on the road to senility and destruction. Recent evidence strongly indicates that small lymphocytes are capable, under appropriate stimulation, of transformation to larger proliferating cells. The circulating small lymphocyte is probably not an "end" cell like the neutrophil, but rather represents a stage in a complex cycle. The concept of aging may therefore not be appropriate to the lymphocyte.

Summary

Tritiated thymidine was administered to C3H mice at frequent intervals by intraperitoneal injection over a 2-week period. About one-third of the circulating small lymphocytes were labeled by this procedure. The animals were then exposed to 208 rads of external irradiation which resulted in a fivefold drop in the total circulating lymphocyte count. The labeled newly formed lymphocytes and the unlabeled older lymphocytes proved to be equally sensitive to radiation. The rate of labeling of peripheral blood lymphocytes was unaffected by a single dose of radiation or by a subsequent dose delivered 4 days later. The decline in the curve of labeling, following the discontinuation of labeling with tritiated thymidine, was the same for irradiated and unirradiated mice. These experiments suggest that the output of new lymphocytes is directly related to the level of circulating small lymphocytes and may possibly depend on transformation of small lymphocytes to large proliferating cells.
TOTAL BODY IRRADIATION EFFECT ON MOUSE LYMPHOCYTES

SUMMARIO IN INTERLINGUA

Thymidina a tritium esseva administrate a muses C3H per frequente injectiones intraperitoneal durante un periodo de 2 septimanas. Circa un tertio del micre lymphocytos circulante esseva marcate per iste procedimento. Postea le animales esseva exponite a 208 rad de irradiation externe lo que resultava in un quintuple declino del numeration de circulante lymphacytos total. Le marcate recentemente formate lymphocytos e le non-marcate lymphocytos de origine anterior se monstrava identicamente sensibile pro le effectos del radiation. Le grado de marcare in le lymphocytos del sanguine peripheric non esseva afficite per un sol close de radiation e non per un subsequente dose administrate 4 dies plus tarde. Le declino in le curva de marcase, post le discontinuation del marcage con thymidina a tritium, esseva le mesme in muses irradiate como in muses non irradiate. Iste experimentos suggere que le rendimento de nove lymphocytos es directemente relationate con le nivello del micre lymphocytos circulante e depende possibilemente del transformation de micre lymphocytos ad in grande cellulas proliferante.

ACKNOWLEDGMENTS

I am indebted to Mrs. Miriam Gray for technical assistance and to Drs. A. A. Axelrad and W. R. Bruce for many helpful discussions.

REFERENCES


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