Plasma Cell Proliferation in the Thymolymphatic Organs of Albino Rats after Total-Body X-Irradiation*

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It is well known that after whole-body exposure to ionizing radiations in a large dose plasma cells are greatly increased in percentage in the thymolymphatic organs (De Bruyn 1948, Murray 1948, Wohlwill and Jetter 1953, Imamura 1959, Keuning et al. 1963). The finding, however, was mainly based on histological observations and there has been very little quantitative information available. In order to obtain some quantitative knowledge regarding the distribution of plasma cells in various lymphoid structures after irradiation, we have undertaken a series of experiments which will be presented below.

Material and Methods

Male albino rats of a subline of the Wistar strain**, about four months old and weighing 200 to 250 gm were used. They were given a single dose of 600 r whole-body X-irradiation from a Shimazu Deep Therapy Machine. The X-ray factors used were 160 kVp, 3 mA, filtration 0.5 mm copper + 0.5 mm aluminium, F.S.D. 30 cm, dose rate 18.94 r per minute.

Twenty-one rats divided into seven groups were sacrificed after one, 3, 5, 7, 10, 14 and 30 days after irradiation, three animals in each group. The thymus, mesenteric lymph nodes and spleen were removed from each animal, fixed in Zenker-formol, and embedded in paraffin. Serial sections 6 micron thick were prepared and studied after hematoxylin and eosin staining.

The differential counting of cells was made on tissue sections by the aid of Abbe's drawing camera (Zeiss). The structures studied were the following: cortex and medulla in the thymus; cortex (and secondary nodules) and medullary cords in the mesenteric lymph nodes; and white and red pulp in the spleen. In general, it was not necessary to further subdivide the structures studied as in the previous report by Fujii of our laboratory (1960, b) because of the marked

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** Rats of this strain were randombred and raised in the animal colony of this laboratory.

simplification of the histological architecture effected by the previous irradiation. A square field of 83.33 $\mu \times$ 83.33 μ was calibrated on paper by means of a stage micrometer, and all nucleated cells in this field were drawn under oil immersion and counted on paper. This procedure was repeated in several different fields on each section and all the cells were counted and added together. The estimation of the number of plasma cells was thus made according to the same criteria as in the previous report (Fujii 1960, b).

Furthermore, the total number of nucleated cells in the thymus, mesenteric lymph nodes and spleen 10 days after irradiation was estimated by Awaya's method (1962), and differential cell counts were made on imprint prescribed in the previous report (Fujii 1960, a). From the values obtained from these observations the absolute number of plasma cells was calculated and compared with that of normal rats.

Result

Differential cell counts in percentage on section of lymphoid organs.

The numerical occurrence of plasma cells after irradiation is illustrated graphically in Figs. 1–5. These figures represent average post-irradiation values in each group. The data in the previous report (Fujii 1960, b) will be interesting when considered as control figures.

1) Thymus.

Within 24 hours after irradiation, the destruction of cortical lymphocytes has left only a few surviving lymphocytes in the medulla. Between the third and 10th days after the irradiation, the shirinkage of thymus continued and the connective tissue proliferation became prominent. From the 10th day on, repopulation with lymphocytes which proceeds slowly outwards from the medulla was observed.

In the control rats, a few plasma cells (constituting 1.04%) were found in the

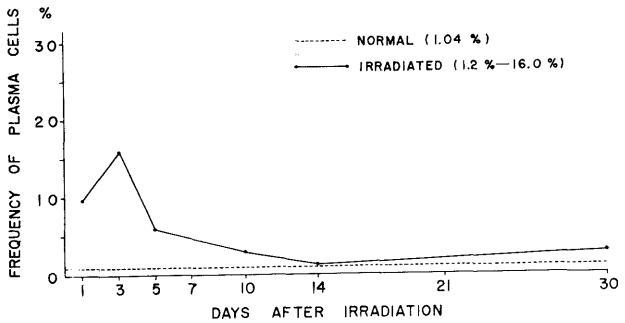

Fig. 1. Medulla of thymus. Frequency of plasma cells in albino rats following 600 r whole-body X-irradiation.

medulla but none in the cortex (Fujii 1960, b). In the medulla of X-irradiated animals the frequency of plasma cells increased to 9.7% on the first day and 16.0% on the third day, followed by a less marked increase after 5th days on (Fig. 1). A very small number of plasma cells (0.1–0.27%) occurred in the cortex also.

2) Mesenteric lymph nodes.

Irradiation produced a massive destruction of lymphocytes. The damage was particularly severe in the Flemming's secondary nodules, resulting in a "nodule-free" period until about 7 days after irradiation, when new nodules began to appear. At this stage, a few immature secondary nodules (bare germinal center of Conway, 1937) consisting of medium-sized lymphocytes in mitosis occurred in the cortical area. These grew rapidly in size and number from the 14th post-irradiation day on and in 30 days came to resemble the typical Flemming's nodules in the control animals. These findings were identical to those described by Imamura in 1959.

The plasma cells are found normally in rat mesenteric lymph nodes, especially

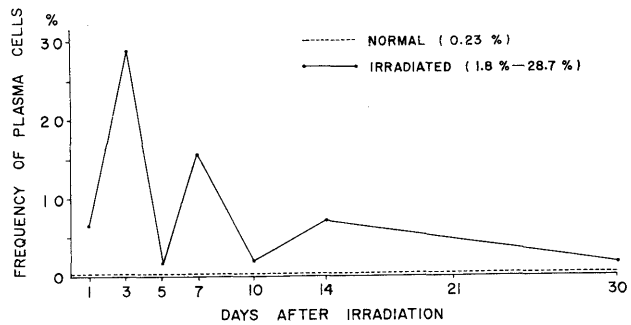

Fig. 2. Cortex of mesenteric lymph nodes. Frequency of plasma cells in albino rats following 600 r whole-body X-irradiation.

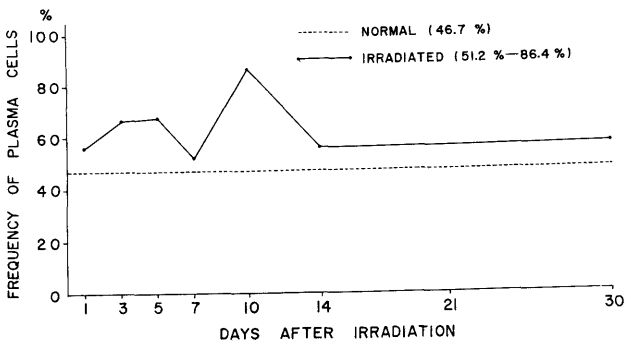

Fig. 3. Medullary cord of mesenteric lymph nodes. Frequency of plasma cells in albino rats following 600 r whole-body X-irradiation.

in the medullary cords (Fujii 1960, b). X-irradiation resulted in a marked increase of plasma cells. The occurrence of plasma cells rose from the base line figure of 0.23% to 1.8–28.7% in the cortex (Fig. 2) and from the base line figure of 46.7% to 51.2–86.4% in the medullary cords (Fig. 3). Such a plasma cell proliferation reached its maximum on the third day in the cortex and at the 10th day in the medullary cords. By this time, the major part of medullary cords was occupied by extensive plasma cell nests. With the progress of regenerative process of the damaged lymph nodes, however, the plasma cells decreased in number gradually.

3) Spleen.

Depletion of white pulp with a decrease in the size of the spleen as a whole was evident following an irradiation. The regenerative process of the secondary nodules in the white pulp was much the same as in the mesenteric lymph nodes.

In the control rats only a few plasma cells were enecountered in the white pulp, especially in the perifollicular zones (0.06%), but a number of these cells in the red pulp is considerably larger, amounting to 1.98% (Fujii 1960, b). In the irradiated rats, plasma cells were predominant in the white pulp as well as

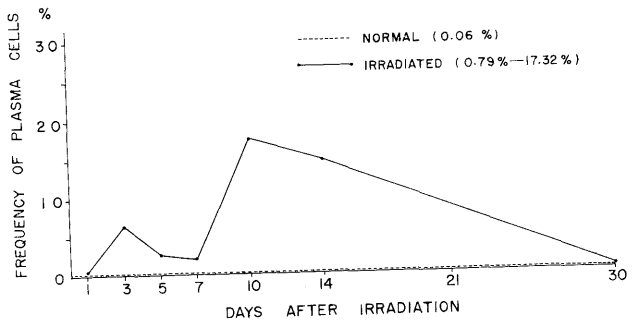

Fig. 4. White pulp of spleen. Frequency of plasma cells in albino rats following 600 r whole-body X-irradiation.

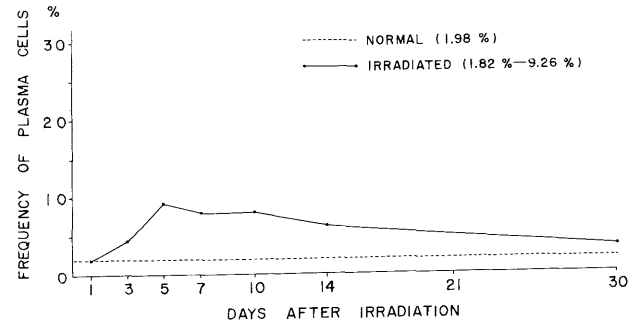

Fig. 5. Red pulp of spleen. Frequency of plasma cells in albino rats following 600 r whole-body X-irradiation.

in the red pulp, increasing from 0.79% to 17.32% in the white pulp and from 1.82% to 9.26% in the red pulp (Fig. 4, 5). After the 10th day on, there has been a gradual decrease in plasma cell number.

Changes in absolute number of plasma cells in lymphoid organs.

The absolute number of plasma cells was estimated on the 10th day by which time the regenerative process of the secondary nodules would be fairly under way and the occurrence of plasma cells would still be found quite richly in the lymphatic tissues. The principal results were listed in Table 1. In all the organs that have been studied, there was a marked increase in the absolute number of plasma cells except in the case of thymus, in spite of a marked decrease in the total number of nucleated cells in these organs.

Table 1. Total number of nucleated cells, plasma cell and lymphocyte counts in thymus, mesenteric lymph nodes and spleen of the normal and X-irradiated rats. Observations on the 10th day following total-body X-irradiation (600 r). Mean ± Standard Error.

Animal group	Organ	Total number of nucleated cells ($\times 10^{-6}$)	Plasma cells		Lymphocytes	
			%	absolute no. ($\times 10^{-6}$)	%	absolute no. ($\times 10^{-6}$)
Normal (7 rats)	Thymus	749±160.9	0.11±0.02	0.8± 0.35	98.7±0.35	739±170.0
	Mesenteric lymph nodes	694± 85.9	1.3 ±0.36	9.0± 3.50	97.2±0.33	657± 85.3
	Spleen	881± 98.3	0.4 ±0.11	3.5± 1.35	89.1±1.54	785±100.5
X-irradiated (7 rats)	Thymus	190± 47.8	0.27±0.06	0.5± 0.25	99.5±0.07	189± 46.8
	Mesenteric lymph nodes	139± 14.2	23.7 ±5.20	33.0±10.30*	74.5±4.90	103± 17.1
	Spleen	315± 34.5	4.8 ±0.82	15.1± 4.23*	82.8±2.21	261± 35.5

* These values are significant at the level of $P<0.01$ compared with those in normal rats.

Discussion

Total-body irradiation with a large dose of X-rays causes a marked increase of plasma cells not only in the medulla of lymph node and the red splenic pulp which are normaly richly supplied with these cells, but also in the cortex of the lymph node, the white splenic pulp and the thymus which either normally contain very little or no plasmacytes at all (Fig. 1–5). The data show that, with the exception of thymus, the increase in occurrence of these cells is the result of absolute increase by an active proliferation, and not simply a relative appearance because of a previously observed massive reduction of lymphocytes caused by the irradiation (Table 1). Although the mechanism and significance of such a plasma cell proliferation is still not yet clarified, yet we have come to consider that the propagation of plasma cell is intimately related to the lymphatic tissue

destruction and to the lymphocyte destruction especially. This phase of the problem was discussed on another occasion (AWAYA et al. 1963).

As regards the relationship between the plasma cells and the other cellular elements following irradiation, opinions of previous investigators are divergent. Murray (1948) claimed that a transformation of lymphocytes into plasma cells takes place as a reaction to the irradiation injury. Latta and Waggener (1954) stated that most of lymphocytes in bone marrow in rats probably became modified into plasma cells after administration of ^{32}P. Keuning et al. (1963) also reported that the periarteriolar lymphocyte sheaths in spleen survived the irradiation and the plasma cells originated from the small lymphocytes of these sheaths. On the other hand, Wohlwill and Jetter (1953) had regarded the reticulum cells as precursor of plasma cells, based upon their observation that the radioresistant reticulum cells became more and more prominent in the lymphatic tissues following irradiation. According to Amano and his colleagues (1948, 1956, 1958), plasma cells are transformed from adventitial cells. From the present study, however, we were unable to obtain information concerning the origin of plasma cells. This problem will be a subject of a future research.

Summary

Young adult male albino rats were exposed to 600 r of total-body X-irradiation and the occurrence of plasma cells was studied on sections made from thymus, mesenteric lymph nodes and spleen. One to 30 days after irradiation, the marked increase in percentage of plasma cells has occurred, reaching its peak during the period of three to 10 days, decreasing in number gradually thereafter. In a quantitative estimation of plasma cell on the 10th day after irradiation, by means of cell counting method used in thymolymphatic tissues (AWAYA, 1962) and of differential cell counts in imprint, there was found an absolute increase of these cells in all organs with the exception of thymus in spite of a marked decrease of total number of nucleated cells.

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