Behavior of Transfused Bone Marrow Cells and Lymphocytes in Irradiated Rats*

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The behavior of the transfused cellular elements of hematopoietic tissues has been pursued in a series of earlier experiments in normal rabbits[1-2] and X-irradiated guinea-pigs.[2-3] The results obtained have demonstrated that the transfused cells exhibit a characteristic behavior in the recipients according to their cell type and may actively proliferate when the animals have been exposed to a large dose of total-body X-irradiation prior to transfusion.

As a continuation of the previous experiments, it was intended in this work to make a comparison of the behavior of the bone marrow cells with that of the lymphocytes after transfusion of these elements into irradiated rats.

MATERIAL AND METHODS

Male Wistar rats weighing around 200 Gm. were exposed in pairs to 600 r total-body X-irradiation.** Immediately following irradiatin, one of each pair received an intravenous injection of a suspension of bone marrow elements or of lymphocytes in physiological saline, while the other which served as the control received an intravenous injection of physiological saline. In every instance, 50,000 units of aqueous penicillin were added to the physiological saline.

In order to standardize the experimental conditions, the same numbers of donors (4–5 male Wistar rats also weighing around 200 Gm.) were used in preparing the cellular suspensions from both the bone marrow and the thymolymphatic organs. The number of nucleated cells injected into one recipient varied from 331 to 1,366 millions.

On the second and the tenth days after irradiation, the animals were killed and total numbers of nucleated cells in the hematopoietic organs were determined, using the methods of cell counting in suspension of nuclei.† In addition, histological examination of various organs was made, after fixing the tissues in ZENKER-formol.

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** Radiation factors were: 160 Kvp, 3 ma., 0.5 mm. Cu+0.5 mm. Al, dose rate in air 12 r. per minute at a distance of 33 cm.

† The methods of counting nucleated cells in the hematopoietic tissues have been described.[4-5]

RESULTS

A. Transfusion of Bone Marrow Cells

On the second day after marrow cells transfusion, bone marrow elements (i.e., myelocytes and erythroblasts) often occurred in small clusters in the dilated lumen

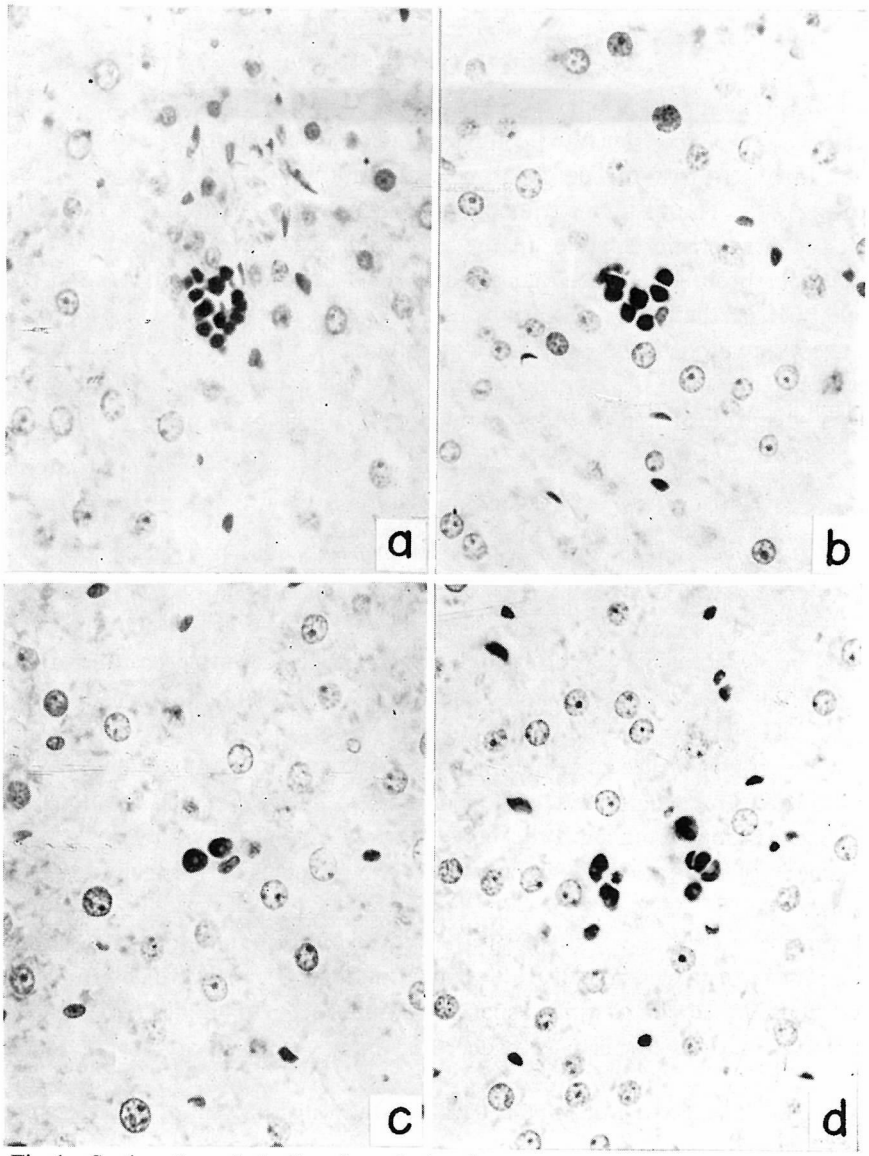

Fig. 1. Sections through the liver from the irradiated rats, showing small collections of bone marrow cells (a-b: erythroblasts; c-d: myelocytes) in the dilated lumen of the sinusoids. Two days after transfusion of bone marrow cells. ×400.

of the sinusoids in the liver of the irradiated hosts (Fig. 1 a-d). In the lungs, lymph nodes, kidneys and adrenals of these animals, however, marrow elements were never seen anywhere until then.

On the tenth day after transfusion, the bone marrow elements, that had once occured in the liver in relatively great numbers on the second day, disappeared almost completely. In the spleen and bone marrow, on the other hand, the populations of myeloid elements appeared to be greatly increased by the tenth day.

By that time the total leukocyte count and the reticulocyte count in the treated rats became significantly higher than the corresponding values in the untreated controls ($P<0.001$) (Table 1).

The most striking effect of the transfusion of marrow cells was on the bone marrow cellularity. By the tenth day after irradiation, there was a marked increase in the cellular density of the femoral bone marrow in the treated rats, as compared with the control values in the untreated rats. The difference between both groups of rats was highly significant ($P<0.001$). Similarly, the estimated values of the total number of nucleated cells in the lymphoid organs, the spleen in particular, were definitely greater in the treated rats than those in the untreated controls (Table 1).

The results of differential cell counts in marrow and spleen imprints on the tenth day after irradiation showed a preponderance of the erythrocytic cell populations over the granulocytic ones in the treated rats, as compared with the control pattern in the untreated rats. Moreover, in the treated rats, there was a concomitant reduction in the percentage of plasma cells, which otherwise would have been greatly increased after irradiation. In the mesenteric lymph nodes, there was no increase in the percentages of myeloid elements after the transfusion of marrow cells (Table 2).

B. Transfusion of Lymphocytes

On the second day after lymphocyte transfusion, there occurred a striking degree of accumulation of lymphocytes, the smaller forms in particular, in the white pulp of the spleen of the irradiated hosts (Fig. 2 a). In contrast, collection of lymphocytes in the splenic white pulp did not occur either in the irradiated rats without lymphocyte transfusion (Fig. 2 b), or in those that had received marrow cell transfusion (Fig. 2 c). In the liver, kidneys and adrenals of the irradiated rats that received lymphocyte transfusion, no accumulation of lymphocytes took place anywhere until the second day.

On the tenth day after lymphocyte transfusion, the lymphatic nodules of the splenic white pulp of the irradiated hosts, that had once regenerated to a considerable extent on the second day, again underwent atrophy, so that there remained relatively small aggregations of actively proliferating, medium-sized lymphocytes in the splenic white pulp (Fig. 3). On the other hand, the cortex of the mesenteric

Table 1.— Summary of the Results of Cell Counting in the Blood and Hematopoietic Tissues, 10 Days after Total-Body X-Irradiation (600 r) and Subsequent Transfusion of Either Bone Marrow Elements or Lymphocytes.

Examinations	Transfusion of Bone Marrow Elements[1]				Transfusion of Lymphocytes[2]			
	Treated	Control	t	P*	Treated	Control	t	P*
Blood								
Total Leukocyte Count	4,790±860	1,430±347	3.210	<0.01	3,180±885	640±98	2.21	<0.05
Reticulocytes Count (%)	+3.6±1.80	−3.7±0.63	3.372	<0.01	−2.6±0.51	−2.9±0.51	0.415	——
Hematopoietc Organs								
Bone Marrows: cellular density ($\times 10^{-6}$/mg)	1.46±0.218	0.80±0.574	5.700	<0.001	0.59±0.13	0.49±0.304	0.586	——
Total Lymphoid Organs[4] ($\times 10^{-6}$)	1,467±236	740±127	2.561	<0.05	1,429±114	964±141	2.32	<0.05
Thymus[4] ($\times 10^{-6}$)	179±56.4	112±36.9	0.918	——	142±31	199±48	0.092	——
Spleen[4] ($\times 10^{-6}$)	821±162	279±45.4	2.80	<0.05	512±70	315±35	2.405	<0.05
Mesenteric Lymph Nodes[4] ($\times 10^{-6}$)	123±12.2	101±20.8	0.966	——	258±33	139±14	3.128	<0.01
Other Lymph Nodes[4] ($\times 10^{-6}$)	344±45.2	234±65.6	1.442	——	516±85	316±42	0.626	——

1 8 rats were used in each group. In the control group two rats died before the 10th day postirradiation. Mean number of nucleated cells transfused into one recipient was $(431\pm64)\times10^6$, ranging from 331 to 491×10^6.

2 8 rats were used in each group. In the treated group one rat and in the control group two rats died before the 10th day postirradiation. Mean number of nucleated cells transfused into one recipient was $(842\pm337)\times10^6$, ranging from 500 to $1{,}360\times10^6$.

3 Designation + indicates an increase and − a decrease as compared with the intial value before irradiation.

4 Tatal number of nucleted cells contained in the whole organ.

* Only significant values of P are indicated.

Table 2.— Summary of the Results of Differential Cell Counts in Imprints of Bone Marrow, Spleen and Mesenteric Lymph Nodes, at 10 Days after Total-Body X-Irradiation and Subsequent Transfusion of either Bone Marrow Elements or Lymphocytes.

Organs and Cell Types	Transfusion of Bone Marrow Cells[1]				Transfusion of Lymphocytes[2]			
	Treated	Control	t	P*	Treated	Control	t	P*
Bone Marrow								
Erythroblasts	43.0±1.65	32.7±2.84	3.72	<0.01	2.95±4.62	22.7±2.68	1.17	——
Total Granulocytes	36.4±2.12	36.5±1.74	0.039	——	28.6±1.42	34.9±3.09	1.92	——
Plasma Cells	1.43±0.11	5.90±1.98	2.42	<0.05	1.99±0.093	7.89±1.95	3.21	<0.01
Lymphoid Cells[3]	15.8±1.61	20.5±2.07	1.84	——	30.8±4.29	27.4±1.41	0.696	——
Spleen								
Lymphocytes	82.3±1.91	82.6±3.03	1.01	——	87.8±1.85	82.7±2.21	1.80	——
Granulocytes	8.29±1.895	7.38±1.00	0.40	——	3.95±1.33	7.65±1.37	1.90	——
Erythroblasts	3.77±0.693	1.23±0.422	2.98	<0.05	0.74±0.151	1.40±2.30	1.93	——
Plasma Cells	2.86±1.328	4.03±1.353	0.53	——	5.19±2.19	4.83±0.82	1.70	——
Mesenteric Lymph Node								
Lymphocytes	82.0±1.89	77.5±6.36	0.74	——	70.8±2.50	74.5±4.90	0.70	——
Granulocytes	1.71±0.635	1.22±0.376	0.63	——	0.49±0.133	0.97±0.305	1.48	——
Plasma Cells	15.7±1.38	19.9±6.02	0.69	——	28.4±2.85	23.7±5.20	0.83	——
Other Cells	0.76±0.295	1.64±0.490	1.76	——	0.35±0.078	0.91±0.250	1.90	——

[1] 8 rats were used in each group. In the control group two rats died before the 10th day postirradiation. Mean number of nucleated cells transfused into one recipient was $(431\pm64)\times10^6$, ranging from 331 to 491×10^6

[2] 8 rats were used in each group. In the treated group one rat and in the control group two rats died before the 10th day postirradiation. Mean number of nucleated cells transfused into onerecipient was $(842\pm337)\times10^6$, ranging from 500 to $1{,}360\times10^6$.

[3] These cells are supposed to be degenerating erythroblasts.

* Ooly significant values of P are indicadte.

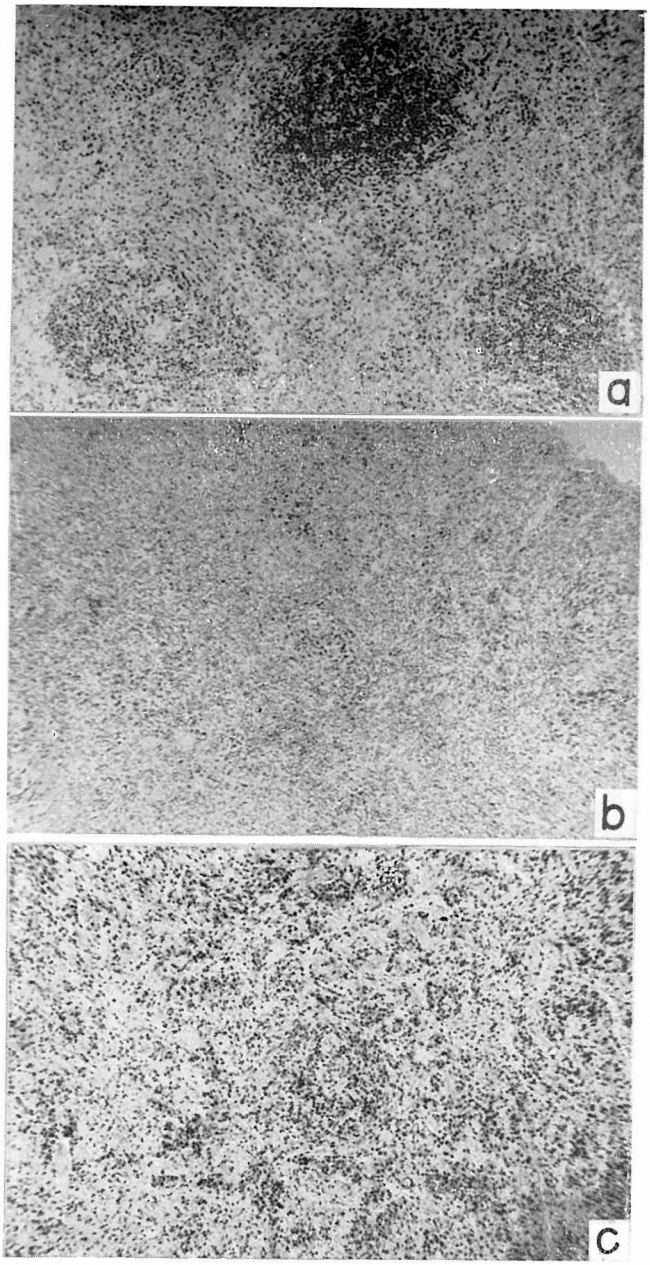

Fig. 2. *a*: Section through the spleen from the irradiated rat that received lymhpocyte transfusion, showing large aggregations of lympocytes in the white pulp. Two days after irradiation and subsequent transfusion. × 100. *b*: Section through the spleen from the irradiated rat without lymphocyte transfusion, showing no collection of lymphocytes in the white pulp. Two days after irradiation. × 100. *c*: Section through the spleen from the irradiated rat that received marrow cell transfusion, also illustrating no collection of lymphocytes in the white pulp. Two days after irradiation and subsequent transfusion. × 100.

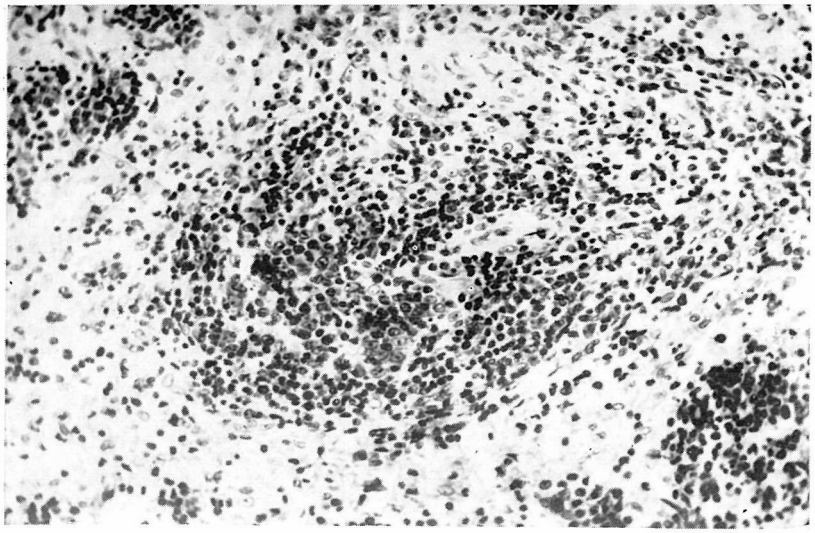

Fig. 3. Section through the spleen from the irradiated rat that received lymphocyte transfusion, showing a small aggregation of actively proliferating, medium-sized lymphocytes in the splenic white pulp. Ten days after irradiation and subsequent transfusion. × 200.

lymph nodes of the treated rats, FLEMMING's secondary nodules in particular, showed a fairly remarkable degree of regeneration by the tenth day after irradiation (Fig. 4 a). The cortical tissue of the mesenteric nodes of the untreated controls showed no indication of regeneration until then. (Fig. 4 b).

Like the transfusion of marrow cells, the transfusion of lymphocytes also caused a marked increase in the number of blood leukocytes, lymphocytes in particular, by the tenth day. However, no simultaneous increase in the percentage of reticulocytes was observed after lymphocyte transfusion.

The effect of lymphocyte transfusion on the bone marrow of the irradiated hosts was essentially different from that of marrow cell transfusion. Namely, after lymphocyte transfusion, there was no increase in the cellular density of bone marrow until the tenth day; whereas after marrow cell transfusion, the bone marrow cellularity was greatly increased by the tenth day (Table 1).

In the lymphoid organs, on the other hand, the total number of nucleated cells was increased to a considerable extent by the tenth day after the transfusion of lymphocytes. This tendency was particularly prominent in the mesenterict lymph nodes (Table 1).

The results of differential cell counts in marrow and spleen imprints indicated no relative increase of the erythrocytic cell population. It should be added, here, that the transfusion of lymphocytes caused a striking reduction of the percentage of plasma cells in the bone marrow, which otherwise would have been greatly increased after irradiation. The transfusion of bone marrow cells also had a similar effect, as

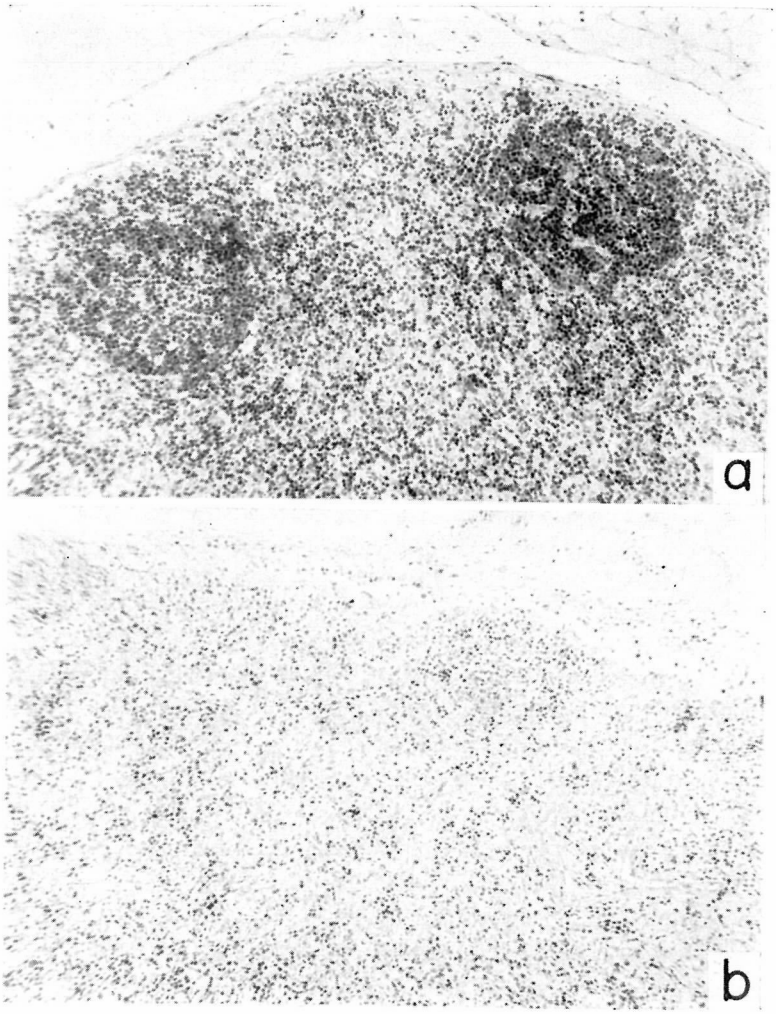

Fig. 4. *a*: Section through the cortex of the mesenteric lymph node from the irradiated rat that received lymphocyte transfusion, showing two large developing secondary nodules. Ten days after irradiation and subsequent transfusion. ×100. *b*: Section through the cortex of the mesenteric lymph node from the irradiated rat without lymphocyte transfusion, which is almost developing secondary nodules. Ten days after irradiation. ×100.

already stated (Table 2).

DISCUSSION

The present experiments demonstrated that, while the transfusion of bone marrow ells caused a rapid regeneration of myeloid tissues of the irradiated hosts, the

transfusion of lymphocytes failed to do so. After lymphocyte transfusion, there was a fairly remarkable degree of regeneration of lymphatic tissues, the splenic white pulp and the cortical nodules of the mesenteric lymph nodes in particular. This is in accord with the observation of CONGDON et al.[6] that the treatment of X-irradiated mice with isologous spleen caused quicker regeneration of splenic white pulp and of lymph nodes than did treatment with isologous bone marrow.

These findings indicate that the chief sites of accumulation and proliferation of the transfused lymphocytes are quite different from those of the transfused bone marrow cells.

In this connection, it is pertinent to refer to the view of YOFFEY et al.,[7] who assumed that lymphocytes produced in excess in the thymolymphatic organs are filtered off in the bone marrow to become stem cells for the development of erythrocytes and granulocytes. If this is really the case, it is to be expected that the transfusion of lymphoctes would effectively accelerate morrow regeneration in irradiated animals.

However, the experiments of CAMPELL and ROSS[8], who used thoractic duct lymphocytes, failed to demonstrate morrow regeneration in lethally irradiated rats after the transfusion of lymphocytes. Recently, VOS et al.[9] have shown that the injection of a large number of homologous lymphoid cells kills X-irradiated mice protected with isologous bone marrow within a week or two. Besides, there are a number of papers reporting similar results.[10-14]

The above-mentioned findings, including ours, do not support the view of YOFFEY et al. that the lymphocytes may transform into mother cells of myeloid elements and, in this way, the transfusion of these cells would accelerate marrow regeneration in irradiated animals.

SUMMARY

After transfusion of either bone marrow cells or lymphocytes into sublethally irradiated rats, the behaviors of both types of cells in the hosts were compared with each other. The results indicated that the chief sites of accumulation and proliferation of the transfused lymphocytes are quite different from those of the transfused marrow cells.

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