THE FATE OF LETHALLY IRRADIATED MICE GIVEN ISOLOGOUS AND HETEROLOGOUS THORACIC DUCT LYMPHOCYTES

B. M. GESNER* AND J. L. GOWANS†

From the Sir William Dunn School of Pathology, Oxford

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IT has been suggested that most of the small lymphocytes which normally enter the blood by way of the main lymphatic vessels are filtered from the blood into the bone marrow where they become " stem " cells from which erythrocytes and granulocytes may develop (Yoffey, 1960). The present experiments were designed to test this view.

It is well known that an injection of isologous bone marrow will save the life of a lethally X-irradiated mouse: the injected cells colonize the mouse and provide it with a new functioning marrow (Ford, Hamerton, Barnes and Loutit, 1956). If lymphocytes can transform into marrow cells than an injection of isologous thoracic duct cells might also be expected to give rise to areas of haemopoiesis and to reduce the mortality from radiation. However, this observation, by itself, would be insufficient to establish that a cellular transformation had occurred. It would be essential to demonstrate with cell-markers that the new bone marrow and circulating blood cells were derived from the injected lymphocytes and were not produced by a recovery of the host's own haemopoietic tissue.

The treatment of X-irradiated animals with lymphocytes obtained from the thoracic duct has given conflicting results. Anderson and Whitelaw (1960) concluded that homologous thoracic duct cells did not influence the recovery of haemopoietic tissue in X-irradiated rats. On the other hand, Delorme (1961) reported that isologous thoracic duct cells did protect some rats against a lethal dose of radiation but the origin of the marrow in the surviving animals was not determined.

There are two reasons for using mice rather than rats in experiments to test the haemopoietic potentialities of lymphocytes. First, a very high proportion of lethally irradiated mice can be saved by the injection of marrow cells. Consequently, if injections of lymphocytes also gave a high degree of protection there would be grounds for supposing that they had induced a rapid haemopoietic recovery. In the rat, the intestinal damage which follows radiation may lead to a high death rate despite treatment with bone marrow (van Bekkum, 1960). It is therefore less justifiable to conclude that measures which give a degree of protection to lethally irradiated rats do so by influencing haemopoiesis. Second, if any " regeneration " of marrow is observed in lethally irradiated mice after

* Postdoctoral Fellow of the Arthritis and Rheumatism Foundation. Present Address: National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

† Member of the External Staff, Medical Research Council.

treatment with mouse thoracic duct cells then its origin can be determined with certainty by using donor cells which carry a chromosome marker (Ford, Hamerton, Barnes and Loutit, 1956).

In the present experiments, lethally X-irradiated mice of a highly inbred strain were injected with large numbers of isologous thoracic duct cells. The survival-time of the animals was recorded and their tissues were examined for the presence of proliferating marrow cells. Since the immunological responses of mice are severely depressed by a lethal dose of X-irradiation (reviewed by Hasek and Lengerová, 1960), the ability of isologous and heterologous (rat) thoracic duct cells to repair this defect was also investigated.

MATERIALS AND METHODS

Animals.—The mice were males belonging to a highly inbred CBA strain. They were 5–8 months old and weighed 20–26 g. The rats belonged to a highly inbred albino strain which is maintained in this laboratory.

X-irradiation.—The mice received 1000 rads of whole-body X-irradiation (Corp, 1957) at the Radiobiological Research Unit, Harwell. This dose kills 98–100 per cent of this strain within 14 days.

Thoracic duct and bone marrow cells.—Lymph was collected from the thoracic duct of mice by the method of Gesner and Gowans (1962). Thoracic duct cells were injected within 14 hr. of their collection from the donor mice. All collections were made within 48 hr. of cannulation ; under these conditions 90–97 per cent of the cells were small lymphocytes and the remainder large and medium lymphocytes. Samples which contained more than an occasional red cell were not used. About 98 per cent of the cells in a 14-hr. collection of lymph were alive as judged by observing their motility at 37° under a phase contrast microscope.

In rats the thoracic duct was cannulated by the method of Bollman, Cain and Grindlay (1948) and the cells were collected in the manner described for mice. About 95 per cent of the cells were small lymphocytes ; the remainder was a heterogeneous collection of large lymphocytes (Gowans, 1957).

Suspensions of bone marrow cells were obtained by flushing out the femur with Krebs-Ringer solution. Thoracic duct and bone marrow cells were concentrated by centrifugation at 150 *g* for 10 min. and resuspended in Krebs-Ringer solution so that 0·4–0·8 ml. contained the desired inoculum for each mouse. Injections of bone marrow or thoracic duct cells were given within 24 hr. after irradiation unless otherwise stated. When killed thoracic duct cells were injected the suspension was first heated at 45° for 10 min. All injections were made into the tail veins.

Histology.—Thymus, lymph nodes and spleen were fixed in Susa or formol-alcohol for staining with haematoxylin and eosin or methyl green-pyronin respectively. The femoral bone marrow was examined in sections stained with haematoxylin and eosin or Giemsa ; femurs were first fixed in formol saline and then decalcified.

RESULTS

Effect of isologous thoracic duct cells

Table I shows that 10 lethally X-irradiated CBA mice, each of which received 8–18·8 $\times$ 10^7 isologous thoracic duct cells, survived no longer than controls which were injected with saline. Eight of the 10 X-irradiated mice which received 1 $\times$ 10^6 isologous bone marrow cells were alive several months after treatment. The remaining 2 mice in this group died from unknown causes 6 days after treatment ; the spleen and bone marrow from these 2 mice showed extensive areas of haemopoiesis. The lymph nodes and spleen of mice which died despite treatment with thoracic duct cells were larger than those of the controls. The thymus was atrophic in both groups.

TABLE I.—*Survival of CBA Mice given an Intravenous Injection of either Isologous Thoracic Duct Cells or Isologous Bone Marrow Cells or Saline within 24 hr. after a Lethal Dose of X-irradiation*

Treatment	Number of cells	Number of survivors at 14 days/ Number injected
Thoracic duct cells	$8{\cdot}0$–$11{\cdot}8 \times 10^7$	0/10
Bone marrow cells	1×10^6	8/10
Saline	—	0/10

Fifteen X-irradiated mice were divided into 3 groups of 5 for a histological study. In the first group each mouse was given approximately 1×10^8 isologous thoracic duct cells and in the second group each received 1×10^6 isologous bone marrow cells. The last group was injected with saline. One animal from each group was killed at 2, 4, 6, 8 and 9 days after treatment and lymph nodes, thymus, spleen and femoral bone marrow were taken for histological examination.

The lymphoid tissues of lethally X-irradiated mice injected with saline showed the extensive cellular damage of severe radiation injury. There was a marked depletion of cells, particularly of small lymphocytes (Fig. 1). The bone marrow was haemorrhagic and contained only a few scattered nucleated cells. No areas of active haemopoiesis remained in the spleen or the bone marrow. These changes were well established by the fourth day and persisted until death.

In animals treated with 1×10^6 isologous bone marrow cells a proliferation of haemopoietic cells became superimposed on the above picture (Figs. 2 and 4). By the 4th day, islands of marrow cells were prominent in the red pulp of the spleen and in the bone marrow. By the 9th day these areas were filled with confluent masses of proliferating haemopoietic cells but the lymph nodes, thymus and the white pulp of the spleen were atrophic and similar in appearance to those of the saline-injected controls.

The lymphoid tissue of mice treated with isologous thoracic duct cells presented a sharp contrast to that of the other groups (Fig. 3). Two days after treatment there was a great increase in the cellularity of the nodes, especially in the cortex, and in the white pulp of the spleen. These changes persisted until death. The thymus remained atrophic. A careful search showed no proliferation of myeloid or erythroid cells in the red pulp of the spleen, elsewhere in the lymphoid tissues, or in the bone marrow (Fig. 5).

The lymph nodes and spleen of mice treated with isologous thoracic duct cells showed a progressive increase in the number of pyroninophilic cells. Large cells with an intensely pyroninophilic cytoplasm, a pale nucleus with a fine chromatin structure and a prominent pyroninophilic nucleolus appeared near the central arteriole in the splenic white pulp and in the cortex of the lymph nodes. In the descriptions which follow, cells with these characteristics will be termed " large pyroninophilic cells " (Figs. 8 and 9). At the edge of the splenic white pulp and sometimes in the red pulp there appeared groups of smaller cells whose pyroninophilic cytoplasm was more abundant relative to the size of the nucleus ; typical plasma cells, which were smaller still, appeared mainly in the medullary cords of the nodes and in the red pulp of the spleen. Mature plasma cells were found in considerably greater numbers in animals treated with isologous thoracic duct cells than in those treated with either saline or isologous bone marrow ; the greatest increase in number was usually seen in the mesenteric lymph node.

Tissues from mice which were injected with 1×10^8 killed isologous lymphocytes had the same appearances as the controls which received saline.

Effect of rat bone marrow and isologous thoracic duct cells

A lethal dose of X-irradiation damages the immunological apparatus of a mouse so that it will accept a homograft or even a heterograft of bone marrow (Lindsley, Odell and Tausche, 1955 ; Nowell, Cole, Habermeyer and Roan, 1956). In 1957 van Bekkum and Vos showed that the ability of a lethally irradiated mouse to reject a graft of rat bone marrow could be restored by a simultaneous injection of isologous lymph node cells ; the beneficial effect of the rat marrow was abolished and the mice died. In the present work thoracic duct cells were substituted for the lymph node cells in the above experiment. The aim was to determine whether thoracic duct cells—that is, lymphocytes which normally enter the blood stream—can re-equip the lymphoid tissues of the irradiated animal and enable it to reject a graft of foreign tissue.

TABLE II.—*Survival of CBA Mice given an Intravenous Injection of Rat Bone Marrow Cells either alone, or together with Isologous Thoracic Duct Cells. The Injections were given within 24 hr. after a Lethal Dose of Irradiation.*

Treatment	Number of cells	Number of survivors at 14 days/ Number injected
Mouse thoracic duct cells + Rat marrow cells	$2{\cdot}5 \times 10^6$ + $2{\cdot}5 \times 10^7$	0/10
Rat Marrow cells	$2{\cdot}5 \times 10^7$	10/10

Table II shows that an intravenous injection of $2{\cdot}5 \times 10^6$ isologous thoracic duct cells completely eliminated the beneficial effect of a simultaneous injection of $2{\cdot}5 \times 10^7$ rat bone marrow cells.

EXPLANATION OF PLATES

FIGS. 1–5.—Tissues from CBA mice 9 days after a lethal dose of whole-body X-irradiation. Intravenous injections of either thoracic duct lymphocytes or bone marrow cells were given on the day of radiation. H. and E.

FIG. 1.—Spleen of untreated mouse. The white pulp is severely damaged and only a few small lymphocytes remain around the central arteriole ($\times 108$).

FIG. 2.—Spleen from mouse given 1×10^6 isologous bone marrow cells. The atrophic white pulp is ringed by haemopoietic tissue which is proliferating in the red pulp ($\times$ 108).

FIG. 3.—Spleen from mouse given 1×10^8 isologous thoracic duct lymphocytes. The white pulp is filled with lymphocytes but the red pulp is atrophic ($\times 108$).

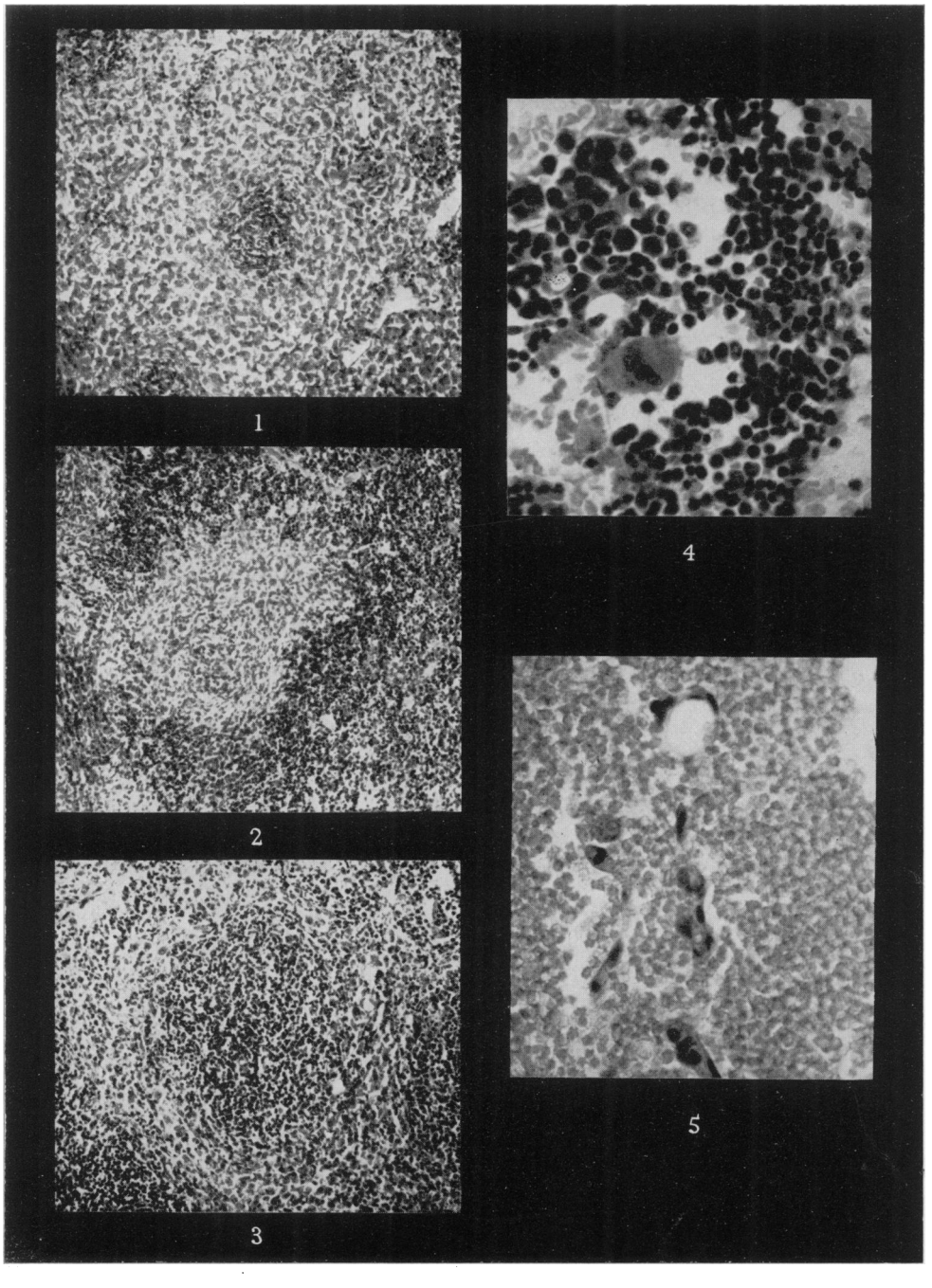
FIG. 4.—Femoral bone marrow from mouse given 1×10^6 isologous bone marrow cells. The marrow is filled with proliferating haemopoietic tissue ($\times 480$).

FIG. 5.—Femoral bone marrow from mouse given 1×10^8 isologous thoracic duct cells. The marrow cavity is filled with erythrocytes. There is no haemopoietic tissue ($\times 480$).

FIG. 6.—Spleen of lethally irradiated mouse 4 days after an intravenous injection of $2{\cdot}5 \times 10^7$ rat marrow cells given on the day of irradiation. The atrophic white pulp is surrounded by proliferating haemopoietic cells. (Methyl green—pyronin $\times 360$.)

FIG. 7.—Spleen of lethally irradiated mouse 4 days after an intravenous injection of $2{\cdot}5 \times 10^7$ rat marrow cells and 1 day after an intravenous injection of $3{\cdot}0 \times 10^7$ isologous thoracic duct lymphocytes. In contrast to the spleen shown in Fig. 6 the white pulp is very cellular and contains many " large pyroninophilic cells " (see text). (Methyl green—pyronin $\times 360$.)

FIGS. 8 and 9.—A high power view of the " large pyroninophilic cells " in the white pulp of the spleen shown in Fig. 7. These cells have an intensely pyroninophilic cytoplasm and a prominent pyroninophilic nucleolus. (Methyl green—pyronin $\times 1500$.)



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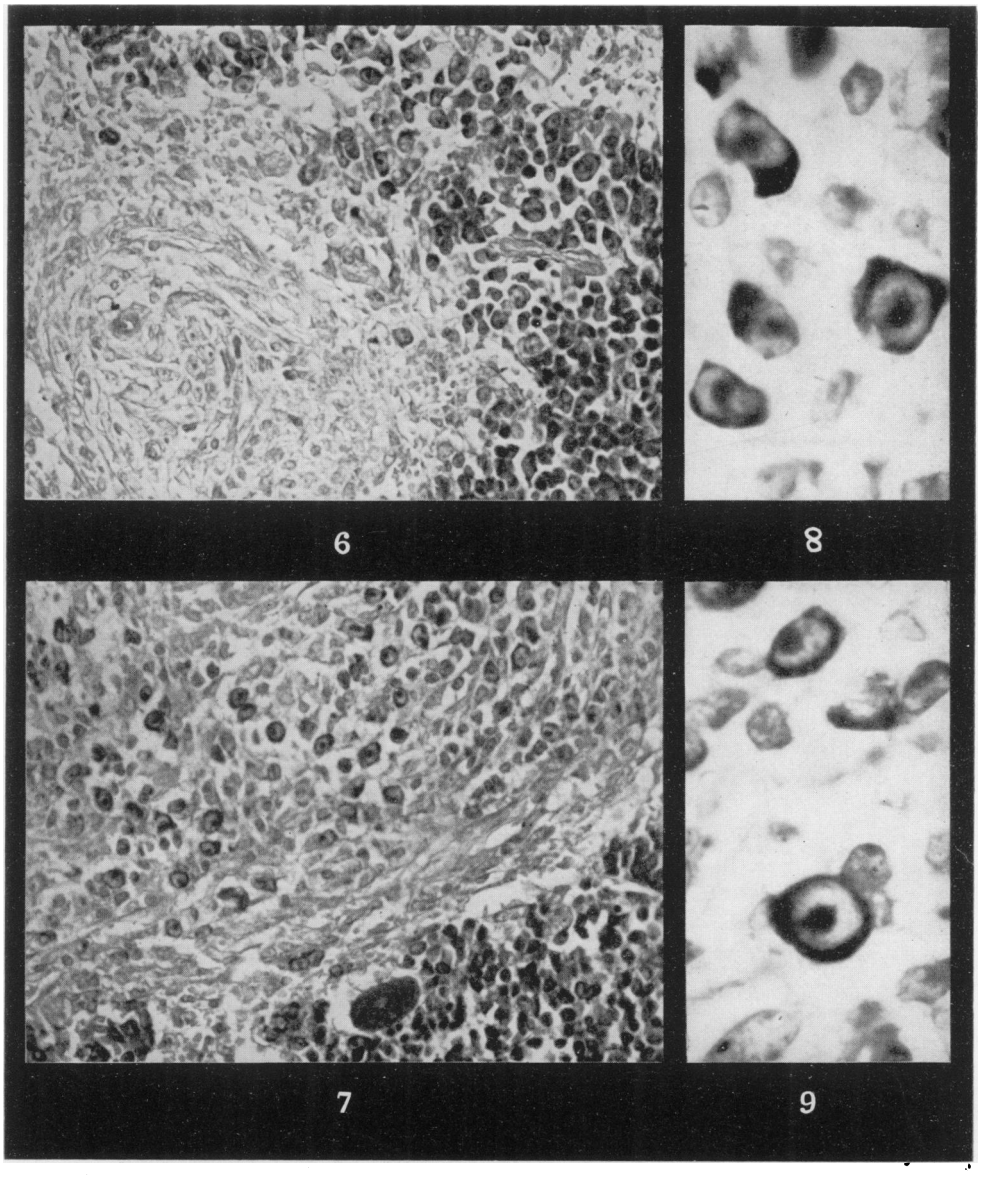

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Nine days after treatment with rat bone marrow alone the red pulp of the spleen and the bone marrow were filled with proliferating erythroid and myeloid cells. In striking contrast, the splenic red pulp of mice receiving the combined injection contained large numbers of mature plasma cells with only a few small islands of marrow cells interspersed among them. The bone marrow was virtually devoid of haemopoietic cells. The cortex of the lymph nodes and the white pulp of the spleen were considerably more cellular than in mice treated with rat marrow alone and contained many large pyroninophilic cells.

An attempt was made to observe in greater detail the histological events which accompanied the elimination of rat marrow in these mice. For this purpose isologous thoracic duct cells were injected into lethally irradiated mice bearing an already established graft of rat bone marrow.

Six mice were X-irradiated and then given $2{\cdot}5 \times 10^7$ rat bone marrow cells on the same day. Three days later they were separated into two groups and the three animals of one group were each injected with 3×10^7 isologous thoracic duct cells. Subsequently, one animal from each group was killed at 24, 48 and 72 hr.

Four days after the injection of rat bone marrow cells alone there were large numbers of marrow cells in the red pulp of the spleen and in the bone marrow but the white pulp of the spleen was atrophic (Fig. 6). Twenty-four hr. after the injection of isologous lymphocytes there was already some destruction of the haemopoietic tissue in the splenic red pulp. The white pulp, however, was very cellular and contained many large pyroninophilic cells (Fig. 7) among which mitotic figures were frequent. Some pyknotic cells were present in the marrow. Two days later the red pulp of the spleen was virtually clear of haemopoietic tissue. There were very few plasma cells in the red pulp although the cellularity and the number of large pyroninophilic cells in the white pulp of the spleen had increased. More pyknotic cells were present in the bone marrow but much haemopoietic tissue still remained.

Effect of rat thoracic duct cells

There is evidence that a lethally irradiated animal may become the victim of a graft-against-host reaction if it is injected with adult lymphoid cells which lack antigens present in the host; the irradiated host cannot destroy the graft, but the graft can mount an immunological attack on the tissues of the host. Thus, treatment with homologous lymph node cells has been found to increase the mortality of mice after X-irradiation (Vos, de Vries, Collenteur and van Bekkum, 1959), and the " secondary disease " which occurs after treatment with homologous or heterologous marrow is thought to be due to an attack on the host by lymphoid

TABLE III.—*The Survival and Spleen Weights of CBA Mice given an Intravenous Injection of either Rat or Isologous Thoracic Duct Cells or Saline within 24 hr. after a Lethal Dose of X-irradiation.*

Treatment	Number of cells	Number of mice	Time of death after treatment (days)	Mean spleen wt. at death (mg.)
Living rat thoracic duct cells	1×10^8	5	6, 6, 6, 7, 7	80·2
Dead rat thoracic duct cells	1×10^8	5	8, 9, 10, 10, 11	19·6
Living mouse thoracic duct cells	1×10^8	5	8, 9, 9, 9, 10	37·7
Saline	—	5	8, 10, 11, 11, 12	22·7

elements derived from the original inoculum of marrow (Barnes, Ford, Ilbery and Loutit, 1958). In the present experiments the ability of lymphocytes to mount a graft-against-host reaction was tested by injecting rat thoracic duct cells into lethally irradiated mice some of which had also received a life-saving dose of isologous marrow cells.

Table III shows that lethally X-irradiated mice which received 1×10^8 rat thoracic duct cells on the day of irradiation died sooner than mice injected with 1×10^8 isologous thoracic duct cells, with killed rat cells or with saline. The mean weight of the spleens from mice treated with rat thoracic duct cells was more than twice that of animals given isologous lymphocytes and almost four times that of the controls. The lymph nodes of mice which were treated with rat thoracic duct cells were larger than those in any other group. The size of the thymus was the same in all groups.

The striking histological change in mice which received rat thoracic duct cells was a rapid proliferation of large pyroninophilic cells in the white pulp of the spleen and in the cortex of the lymph nodes. This proliferation greatly exceeded the similar change seen in mice receiving isologous lymphocytes. At death, many mature plasma cells were seen in the red pulp of the spleen.

TABLE IV.—*Survival of CBA Mice given an Intravenous Injection of Isologous Bone Marrow Cells either alone or together with Rat Thoracic Duct Cells. The Injections were given within 24 hr. after a Lethal Dose of Irradiation*

Treatment	Number of cells	Number of survivors at 14 days/ Number injected
Rat thoracic duct cells	$1 \cdot 0$–$1 \cdot 36 \times 10^8$	
+	+	1/10
Mouse Marrow cells	1–6×10^6	
Mouse marrow cells	1–6×10^6	9/10

Table IV shows that treatment with rat thoracic duct cells virtually eliminated the beneficial effects of a simultaneous injection of isologous marrow cells. This effect could only be regularly achieved if 1×10^8 or more rat lymphocytes were given. This is in contrast to the small number of isologous lymphocytes which were invariably effective in abolishing the protective effect of rat marrow in lethally irradiated mice.

Nine days after the combined treatment there was no haemopoietic tissue in the bone marrow or in the red pulp of the spleen. The architecture of the spleen was more disorganized than in any other group of mice in these experiments. The red pulp was haemorrhagic and contained few plasma cells and the white pulp, which was poorly demarcated, contained a mass of proliferating, large pyroninophilic cells. The lymph nodes also contained many large pyroninophilic cells and the normal follicular structure was noticeably disrupted.

DISCUSSION

It has been suggested that a migration of lymphocytes from the blood to form " stem " cells in the bone marrow accounts for the disappearance from the blood of a large part of the normal daily output of lymphocytes from the main lymph ducts (Yoffey, 1960). The present experiments provide no support for even a

small-scale process of this kind in the mouse. The intravenous injection of 1×10^8 isologous thoracic duct cells did not prolong the life of lethally X-irradiated mice, nor did it lead to any haemopoiesis in their tissues over a period of 9–12 days. Under the same condition 17 out of 20 X-irradiated mice survived after treatment with 1×10^6 isologous bone marrow cells, and the remainder showed histological evidence of extensive haemopoiesis. The failure of 1×10^8 isologous thoracic duct cells to give rise to any histological or clinical signs of haemopoiesis makes it unlikely that any cell present in thoracic duct lymph at a concentration of more than 1 per cent can differentiate into a marrow stem cell. This figure becomes even smaller when it is considered that only a fraction of a marrow inoculum consists of cells which would be able to proliferate after transplantation. A figure of 1 per cent would exclude not only the small lymphocyte, but also the group of larger lymphocytes which comprised 3–10 per cent of the thoracic duct cells injected in these experiments. It does not exclude the presence in the lymph of very rare cells with haemopoietic potentialities ; nor does it deny the presence in normal blood of cells capable of repopulating the marrow of irradiated animals (Popp, 1960), but it suggests that these probably do not enter the blood by way of the thoracic duct.

The failure to observe any functional connection between the activity of thoracic duct lymphocytes and marrow in irradiated mice was complemented by the histological observation that quite different areas became repopulated with cells after the two kinds of treatment. Injections of thoracic duct cells increased the cellularity of the lymph nodes and the white pulp of the spleen, while bone marrow cells gave rise to a massive proliferation in the bone marrow and in the splenic red pulp. No change was seen in the thymus after either treatment. Micklem and Ford (1960), using a chromosome marker, showed that lymph node cells which were injected into lethally irradiated mice proliferated in the lymph nodes but not to any significant extent in the thymus or bone marrow, a result which parallels the histological findings in the present study.

It could be objected that the lethally irradiated mouse may not provide the correct environment for the differentiation of lymphocytes into marrow stem cells. It can only be said that this environment will allow transplanted lymphoid cells to establish themselves and proliferate (Micklem and Ford, 1960) while haemopoietic cells rapidly give rise to a functioning bone marrow. The way in which grafts of bone marrow survive in certain lethally irradiated hosts is itself evidence that the bone marrow is a self-sustaining tissue and does ont depend for its survival on a continuous large-scale influx of lymphocytes from the blood. Thus, the extremely atrophic lymphoid tissue of homologous radiation chimeras with " secondary disease " does not apparently prejudice the survival of the graft of bone marrow (Ilbery, Koller and Loutit, 1958).

The findings in these experiments are apparently at variance with those of Delorme (1961) who found that isologous thoracic duct cells protected 43 per cent of rats against a lethal dose of X-irradiation. This discrepancy is probably due to differences in the sensitivity of mouse and rat tissues to X-irradiation. The death of mice which receive a just-lethal dose of radiation is closely connected with the destruction of the bone marrow. For example, van Bekkum and Vos (1957) found that all their lethally irradiated mice recovered after receiving as few as 1×10^5 isologous bone marrow cells. The recovery of lethally irradiated mice should therefore be a sensitive indicator of the haemopoietic potentialities

of cells injected into them. On the other hand, Delorme (1961) found that only 54 per cent of irradiated rats survived after receiving large inocula of isologous marrow cells. The " intestinal syndrome " which develops in lethally irradiated rats and which is not influenced by treatment with bone marrow (van Bekkum, 1960) may have accounted for this high death rate. The recovery of some irradiated rats after an injection of thoracic duct cells might well be due to an amelioration of the intestinal injury by, for example, the rapid re-equipment of the animals' lymphoid tissue. The observation that shielding the small intestine reduces the mortality of rats after a lethal dose of radiation (Swift and Taketa, 1956), together with the results of the present experiments, makes an explanation of this kind very likely. But the point can only be settled by determining whether the new marrow in irradiated rats treated with thoracic duct cells is donor or host in origin.

Whether or not lymphocytes play any part in haemopoiesis there is no doubt that they possess immunological activity. Wesslén (1952*a*, *b*) was the first to show that thoracic duct cells from immunized animals continued to produce antibody *in vitro* and could mediate an adoptive transfer of tuberculin hypersensitivity. More recently it has been shown that injections of thoracic duct cells from genetically appropriate donors will induce runt disease in newborn rats (Anderson, Delorme and Woodruff, 1960 ; Billingham, Brown, Defendi, Silvers and Steinmuller, 1960), a lethal graft-against-host reaction in normal adult F_1 hybrid rats, and the breakdown of a long-standing homograft of skin in tolerant rats (Gowans, Gesner and McGregor, 1961). The present experiments again illustrate the activity of thoracic duct cells in the destruction of grafts of foreign tissue and in graft-against-host reactions. Thus, an injection of normal isologous lymphocytes enabled a lethally irradiated mouse to destroy a graft of rat bone marrow, while rat lymphocytes not only eliminated a graft of isologous bone marrow but also hastened the death of irradiated mice which received no other treatment. The mechanism by which lymphocytes bring about these destructive effects is not known although there is some evidence that circulating antibody can eliminate a graft of foreign bone marrow (Garver and Cole, 1961).

In the present study no attempt was made to determine which cells in thoracic duct lymph were responsible for the immunological effects although the choice clearly lies between the small lymphocyte, the large lymphocyte, or both cells in combination. In the lethal wasting disease produced by injecting parental thoracic duct cells into F_1-hybrid rats it has been shown that the small lymphocyte in thoracic duct lymph is probably the immunologically active cell (Gowans *et al.*, 1961 ; 1962).

The destruction of grafts of either isologous or heterologous marrow which followed the injection of thoracic duct cells into irradiated mice was always attended by the appearance and proliferation in the white pulp of the spleen of large cells with an intensely pyroninophilic cytoplasm, a pale nucleus and a prominent nucleolus. They also appeared in large numbers when rat lymphocytes alone were injected into irradiated mice and in small numbers when isologous lymphocytes alone were given. It seems very likely that in all these experiments the large pyroninophilic cells developed from donor lymphocytes (either large, small or both) which had migrated from the blood into the splenic white pulp. Chromosome studies have made it quite certain that when rat thoracic duct cells alone are injected into lethally irradiated mice many of the large pyroninophilic cells

which appear in the spleen are derived from the donor's small lymphocytes (Ford and Gowans, in Gowans, 1962). The possible contribution of rat large lymphocytes to the reaction in the mouse spleen has not yet been detemined.

Large pyroninophilic cells with the morphological characteristics of those seen in the present experiments have been described in a number of immunological reactions. For example, they have been observed in the spleen and lymph nodes during antibody formation (Fagraeus, 1948 ; Leduc, Coons and Connolly, 1955), in the regional lymph nodes draining a homograft of skin (Scothorne, 1957), and in the spleen during a graft-against-host reaction in rats (Gowans *et al.*, 1961 ; 1962). In the last of these examples there is evidence that they were derived from the donor's small lymphocytes. Their origin in the other situations is uncertain although the possibility that they were derived originally from small lymphocytes must now be considered (Gowans, 1962). The term " haemocytoblast " (Fagraeus, 1960) which has been suggested for this class of large pyroninophilic cell has been deliberately avoided since it implies a potentiality for development into cells which circulate in the blood, and this has not been demonstrated.

The fate of the large pyroninophilic cells was not determined. The histological picture in some experiments suggested that a proportion of them may have migrated into the splenic red pulp as they matured into plasma cells. A migration of this kind has been inferred by Congdon and Makinodan (1961) from the histological changes which occur in the mouse spleen during antibody formation. However, in other experiments few mature plasma cells appeared despite an intense proliferation of large pyroninophilic cells in the white pulp of the spleen and it is not clear whether the progeny of the dividing cells were destroyed or whether they passed out of the spleen into the blood, perhaps as lymphocytes.

SUMMARY

The intravenous injection of large numbers of isologous thoracic duct lymphocytes into lethally irradiated mice did not reduce the mortality from radiation, nor did it lead to any haemopoiesis in the spleen or the bone marrow.

The injection of isologous lymphocytes restored the ability of lethally irradiated mice to reject a graft of rat bone marrow. Lethally irradiated mice which received rat thoracic duct lymphocytes died more quickly than untreated controls or mice receiving isologous lymphocytes. An injection of rat lymphocytes also eliminated the beneficial effect of treatment with isologous bone marrow. These reactions in lethally irradiated mice were attended by the appearance and proliferation in the splenic white pulp of large cells with an intensely pyroninophilic cytoplasm, a pale nucleus and a prominent nucleolus. These cells probably developed from the injected lymphocytes.

The experiments do not support the idea that lymphocytes can transform into marrow " stem " cells. They illustrate the immunological activity of lymphocytes.

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