BONE MARROW ORIGIN OF IMMUNOLOGICALLY COMPETENT LYMPHOCYTES IN THE RAT*

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The ability of small lymphocytes to cause graft vs. host reactions in rodents demonstrates that cells of this type are immunologically competent (1). However, no information is provided about the origin of small lymphocytes or the stage at which they, or their precursors, acquire the ability to initiate immunological responses.

Experiments using irradiated mice indicate that immunologically competent cells can develop from precursors in adult bone marrow. Injections of bone marrow cells can save the lives of mice exposed to a lethal dose of whole body X- or γ-irradiation (2, 3). Cells from donor marrow correct the immunological deficiency in such animals if they have either an intact thymus or thymus graft (4, 5). Similarly, cells from a variety of tissues, including bone marrow, can contribute competent cells to spleen and lymph nodes of sublethally irradiated mice by a thymus-dependent mechanism (6–9). The results suggest that marrow contains progenitors of immunologically competent cells but it cannot be concluded that the competent cells are small lymphocytes.

From a study in which normal, newborn F_1 hybrid rats were inoculated with parental strain bone marrow cells, Goldschneider and McGregor (10) concluded that circulating, immunologically competent lymphocytes can develop from myeloid precursors. Thoracic duct cells (presumably small lymphocytes) obtained from the inoculated animals in adult life were found to have the immunological capability of the marrow donor. The present experiments provide additional evidence that rat bone marrow can generate immunologically competent small lymphocytes. They suggest that lymphocytes capable of initiating a lethal graft vs. host reaction can develop from cells normally resident in bone marrow and that these lymphocyte precursors are either absent, or present in only small numbers, in the peripheral lymphocyte pool.

Methods

The general plan of the experiments was as follows. First, a comparison was made of the ability of bone marrow cells and thoracic duct lymphocytes from adult Lewis rats to cause a systemic graft vs. host reaction (homologous disease) in lightly X-irradiated adult members

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of the (Lewis × BN)F_1 hybrid cross. Second, bone marrow cells or lymphocytes from Lewis donors were injected into normal, nonirradiated, newborn (Lewis × DA)F_1 hybrid rats (intermediate F_1 hosts). 6–18 wk later, the inoculated animals donated thoracic duct lymphocytes to irradiated recipients belonging to either the (Lewis × BN)F_1 hybrid cross or the (DA × BN)F_1 hybrid cross (secondary F_1 hosts). In each case, the secondary F_1 host was sensitized to (Lewis × DA)F_1 tissues before irradiation. This procedure protected the animal from homologous disease caused by the intermediate F_1 host's own lymphocytes. Development in the secondary host of transient erythema, dermatitis, and progressive body wasting, beginning 8 days or longer after injection, was taken as evidence of a graft vs. host reaction initiated by small lymphocytes derived from cells originally present in the Lewis bone marrow inoculum.

TABLE I

Effect of an Intravenous Injection of Lewis Bone Marrow Cells or Thoracic Duct Lymphocytes on (Lewis × BN)F_1 Hybrid Rats

Donor			Recipient*		
Treatment	Inoculum	No. cells × 10^8	No. inoculated	No. ill	No. killed
Normal	Thoracic duct lymphocytes	5	5	5	5
		2	10	10	10
		1	14	12	11
		0.5	22	13	8
Normal	Bone marrow cells	50	10	9	1
		25	8	4	0
		12.5	8	3	0
Depleted of lymphocytes‡	Bone marrow cells	100	7	0	0
		50	8	0	0

* Female rats 60–90 g body weight. The animals, sensitized to (Lewis × DA)F_1 tissues were exposed to 350 rads of whole body X-irradiation 24 hr before injection.

‡ Male and female rats 180–280 g body weight depleted of lymphocytes by drainage from a thoracic duct fistula for 5 days immediately before sacrifice.

Animals.—The subjects of these experiments were male and female Lewis rats, and female members of the F_1 hybrid cross between Lewis rats and rats belonging to either the DA or BN strains. Female (DA × BN)F_1 hybrid rats also were used in one experiment.

Preparation of Cell Suspensions.—Tissues were removed from ether-killed rats. Cells were expressed from spleen and thymus by teasing the tissues with fine forceps in tissue culture medium 199 (11) containing 1 unit of heparin/ml. Medium 199 containing the same concentration of heparin was used to flush cells from the marrow cavity of femurs and tibias.

Lymphocytes were obtained from the thoracic duct of unanesthetized rats. The method used in cannulating the thoracic duct, and the postoperative management of the rats, has been described in detail by Gowans and Knight (12). Lymph from freshly cannulated animals was collected at 4°C for periods of 8–16 hr into sterile flasks containing 5 ml of Krebs-Ringer solution, 100 units of heparin and 500 μg of streptomycin.

Cell suspensions were filtered through several layers of surgical gauze to remove tissue

debris and fibrin clots. The cells were then centrifuged for 10 min at 100 *g* and resuspended in heparin-free medium at a concentration of 25×10^7 cells/ml for injection into newborn rats, and 10^6–10^8 cells/ml for injection into adult animals. More than 95% of cells prepared in this way were viable as judged by their ability to exclude trypan blue.

Intravenous Injection.—Bone marrow cells and thoracic duct lymphocytes were injected through a No. 30 needle into the lateral tail vein of unanesthetized newborn rats. Blanching of the vein, transient cyanosis, and respiratory distress testified to the success of intravenous injection. Adult rats were anesthetized with ether and injected by the same route as newborn rats.

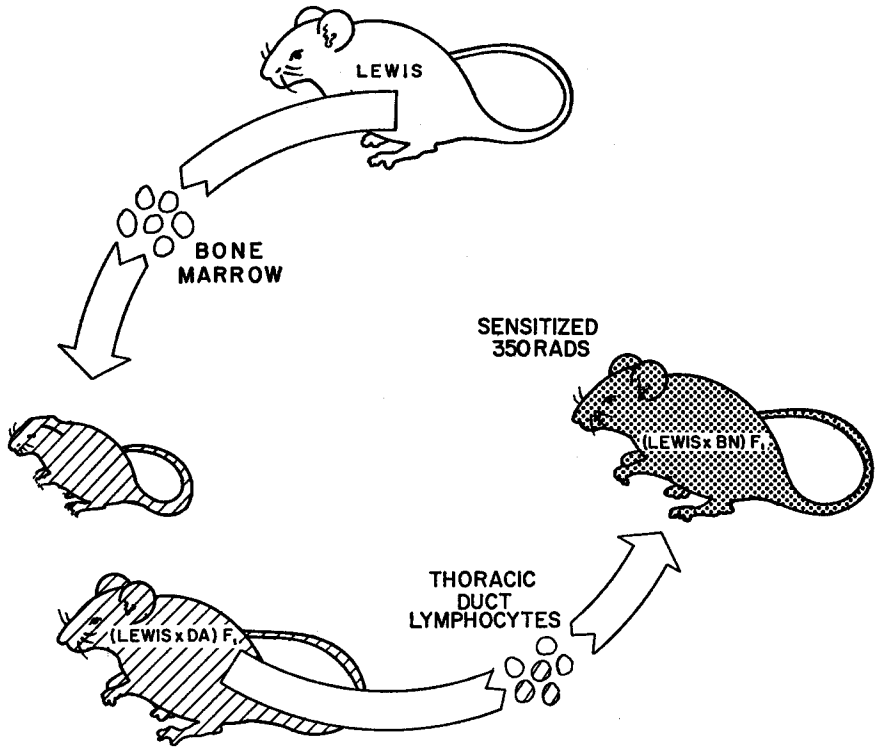

FIG. 1. Schematic representation of assay for immunologically competent parental strain lymphocytes in parental F_1 chimeras.

Induction of Tolerance.—Lewis rats were rendered immunologically tolerant by injecting the animals intravenously on the day of birth with 5×10^7 bone marrow cells from adult donors belonging to the (Lewis × DA)F_1 hybrid cross. 3 wk later, the inoculated animals received a full thickness (Lewis × DA)F_1 skin graft. Survival of the graft in excellent condition for longer than 50 days was taken arbitrarily as evidence of tolerance.

Sensitization.—(Lewis × BN)F_1 rats and (DA × BN)F_1 rats, 40–60 g body weight, were sensitized by two intraperitoneal injections of a mixture of 2×10^8 (Lewis × DA)F_1 hybrid spleen cells and thymocytes, 6 days apart. The second injection was given 5 days before the animals were exposed to X-irradiation.

X-Irradiation.—Groups of two to four unanesthetized rats in ventilated polystyrene boxes were exposed to 350 rads of whole body X-irradiation. X-rays were delivered from a General Electric deep therapy unit of 250 kv operating at 15 ma with added filtration of 0.25 mm of Cu and 1 mm of Al. The beam had a half-value layer of 1.5 mm of Cu. The distance from target to midline of the animals was 50 cm and the dose rate was 52 rads/min.

RESULTS

Immunological Capability of Cells in Bone Marrow and Thoracic Duct Lymph.—Rat bone marrow cells are much less effective than thoracic duct lympho-

TABLE II

Effect of an Intravenous Injection of 10^8 (Lewis × DA)F_1 Hybrid Thoracic Duct Lymphocytes on (Lewis × BN)F_1 Hybrid Rats

Treatment of (Lewis × DA)F_1 donor*		Fate of (Lewis × BN)F_1 recipient‡		
Neonatal inoculum	Age when cannulated	No. inoculaed	No. ill	No. killed
	wk			
Normal bone marrow	6	5	5	5
	10	5	5	5
	11	10	10	8
	12	2	2	1
	14	4	4	4
	18	3	3	3
Lymphocyte-deficient bone marrow§	8	5	5	4
	11	5	3	2
	14	4	4	4
Not inoculated	8	4	0	0
	9	6	0	0
	12	5	2	0
	18	8	0	0

* Male and female rats inoculated at birth with 5×10^7 Lewis bone marrow cells.

‡ Female rats 60–90 g body weight. The animals, sensitized to (Lewis × DA)F_1 tissues, were exposed to 350 rads of whole body X-irradiation 24 hr before injection.

§ From male and female rats depleted of lymphocytes by drainage from a thoracic duct fistula for 5 days immediately before sacrifice.

cytes in causing graft vs. host reactions. Table I shows that 5×10^7 bone marrow cells from normal Lewis rats killed only 1 of 10 lightly X-irradiated, young adult members of the (Lewis × BN)F_1 hybrid cross. In contrast, 10^6 Lewis lymphocytes were lethal for the majority of similar recipients.

The weak immunological capability of rat bone marrow probably is invested in its content of circulating small lymphocytes. Bone marrow cells from rats depleted of small lymphocytes by chronic drainage from a thoracic duct fistula

(13) are even less effective than bone marrow cells from normal donors in causing homologous disease in F_1 hybrid recipients. Table I indicates that as many as 10^8 bone marrow cells from lymphocyte-depleted Lewis donors had no obvious effect on irradiated (Lewis × BN)F_1 recipients.

Origin of Immunologically Competent Lymphocytes.—Although rat bone marrow contains only a few immunologically competent lymphocytes, it is a potent source of their precursors. This conclusion emerges from experiments illustrated in Fig. 1. Newborn (Lewis × DA)F_1 rats were inoculated with 5×10^7 bone marrow cells from either normal, or lymphocyte-depleted, adult Lewis donors. Thoracic duct lymphocytes obtained from the inoculated animals later

TABLE III

Effect of an Intravenous Injection of 10^8 Lewis and (Lewis × DA)F_1 Hybrid Thoracic Duct Lymphocytes on (Lewis × BN)F_1 Hybrid Rats

No. of lymphocytes inoculated × 10^6			Fate of (Lewis × BN)F_1 recipient*		
Normal Lewis	Normal (Lewis × DA)F_1	Marrow-inoculated (Lewis × DA)F_1‡	No. inoculated	No. ill	No. killed
—	—	100	29	29	26
—	50	50	6	2	1
10	90	—	7	7	7
5	95	—	8	8	8
2	98	—	8	8	8
1	99	—	10	10	10
0.5	99.5	—	6	2	1
0.2	99.8	—	7	0	0
—	100	—	23	2	0

* Female rats 60–90 g body weight. The animals, sensitized to (Lewis × DA)F_1 tissues, were exposed to 350 rads of whole body X-irradiation 24 hr before injection.

‡ Male and female rats inoculated at birth with 5×10^7 Lewis bone marrow cells.

in life were injected intravenously into sensitized and X-irradiated adult members of the (Lewis × BN)F_1 hybrid cross.

Table II indicates that 10^8 lymphocytes from F_1 rats receiving normal Lewis bone marrow cells were lethal for all but three of the secondary F_1 hosts. Even lymphocytes from donors inoculated at birth with lymphocyte-deficient bone marrow killed the majority of recipients. In contrast, 10^8 lymphocytes from normal, uninoculated (Lewis × DA)F_1 donors caused a nonfatal, graft vs. host reaction in only 2 of 23 irradiated (Lewis × BN)F_1 hybrid rats.

The immunological performance of 10^8 thoracic duct lymphocytes from (Lewis × DA)F_1 rats inoculated at birth with Lewis bone marrow was measured against the performance of an equal number of lymphocytes in mixtures prepared from normal Lewis donors and normal, uninoculated (Lewis × DA)F_1

donors. Table III shows that lymphocytes from F_1 recipients of Lewis bone marrow had approximately the same effect on X-irradiated (Lewis × BN)F_1 hybrid rats as a mixture containing 10^6 normal Lewis lymphocytes. Mixtures containing more than 10^6 Lewis lymphocytes caused a particularly vicious graft vs. host reaction as testified by rapid onset of illness and early death of the host.

Results of these experiments suggest that the peripheral pool of circulating

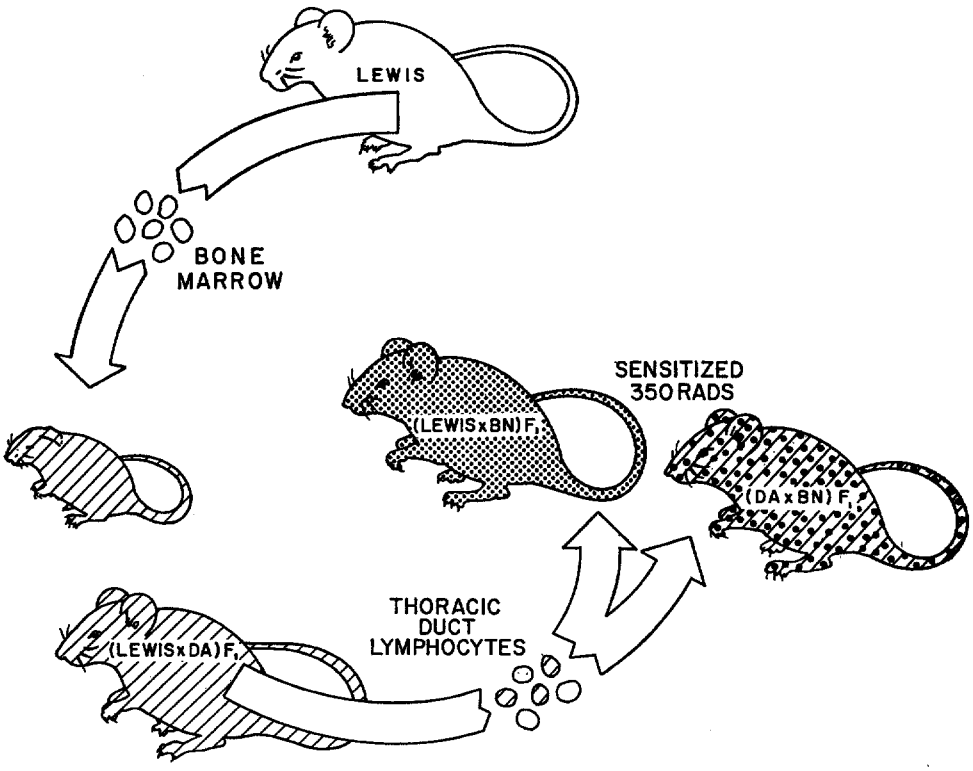

FIG. 2. Schematic representation of assay for immunologically competent lymphocytes in parental F_1 chimeras.

small lymphocytes in F_1 rats inoculated with parental strain bone marrow contains approximately 1% of lymphocytes derived from precursors in the marrow inoculum. This calculation is based on the premise that only Lewis strain lymphocytes engage in the graft vs. host reaction, and that the performance of these lymphocytes is unaffected by their passage through the intermediate F_1 host. The intermediate F_1 host's own lymphocytes probably have little or no effect on X-irradiated (Lewis × BN)F_1 rats. Results in Table III support this view. Thus, 10^8 thoracic duct lymphocytes from normal, uninoculated (Lewis

$\times$ DA)F_1 donors had no obvious effect on 21 of 23 irradiated (Lewis $\times$ BN)F_1 recipients.

It could be objected that lymphocytes of F_1 rats inoculated at birth with parental strain bone marrow acquire an increased capacity to react against tissue isoantigens of the secondary F_1 host. This unlikely possibility was studied in the experiment illustrated in Fig. 2. Newborn (Lewis $\times$ DA)F_1 rats were inoculated with 5×10^7 bone marrow cells from normal Lewis donors. At varying intervals later, the inoculated animals donated thoracic duct lymphocytes to X-irradiated adult rats belonging to F_1 hybrid crosses which shared a BN parent. In each case, the secondary F_1 hosts were inoculated with (Lewis $\times$ DA)F_1 lymphoid cells before irradiation. This procedure sensitized (Lewis $\times$

TABLE IV

Effect of an Intravenous Injection of 10^8 (Lewis $\times$ DA)F_1 Hybrid Thoracic Duct Lymphocytes on (Lewis $\times$ BN)F_1 and (DA $\times$ BN)F_1 Hybrid Rats

Treatment of (Lewis $\times$ DA)F_1 donor*	Recipient strain	Fate of recipient‡		
		No. inoculated	No. ill	No. killed
Normal bone marrow	(Lewis $\times$ BN)F_1	29	29	26
Lymphocyte-deficient bone marrow§	(Lewis $\times$ BN)F_1	14	12	10
Normal bone marrow	(DA $\times$ BN)F_1	8	1	0
Not inoculated	(Lewis $\times$ BN)F_1	23	2	0

* Male and female rats inoculated at birth with 5×10^7 Lewis bone marrow cells.

‡ Female rats 60–90 g body weight. The animals, sensitized to (Lewis $\times$ DA)F_1 tissues, were exposed to 350 rads of whole body X-irradiation 24 hr before injection.

§ From male and female rats depleted of lymphocytes by drainage from a thoracic duct fistula for 5 days immediately before sacrifice.

BN)F_1 rats to isoantigens of the intermediate F_1 host, whereas (DA $\times$ BN)F_1 rats were sensitized to isoantigens common to the intermediate host and the marrow donor.

Table IV shows that lymphocytes from recipients of Lewis bone marrow were especially toxic for irradiated rats belonging to the (Lewis $\times$ BN)F_1 hybrid cross. Failure of lymphocytes from marrow inoculated F_1 donors to cause a lethal reaction in (DA $\times$ BN)F_1 recipients argues against the possibility that the donor's own lymphocytes acquire an increased ability to react against BN tissue isoantigens. The results are consistent with the view that homologous disease in the secondary F_1 host is caused mainly (perhaps exclusively) by lymphocytes derived from cells in the Lewis bone marrow inoculum.

Experiments Using Lymphocytes from Immunologically Tolerant Rats.—In adult rats, the peripheral pool of circulating small lymphocytes contains mature, immunologically competent cells but, when compared with bone marrow, the

pool is relatively deficient in their precursors. Results of experiments illustrated in Fig. 3 support this view. The experiments exploit two characteristics of lymphocyte populations in immunologically tolerant rats. First, lymphocytes of such animals are less effective than those of normal donors in their ability to respond to tolerated antigens (14–18). Second, the defect in immunological performance is specific for antigens to which the donor is tolerant (15, 16, 18). In the present

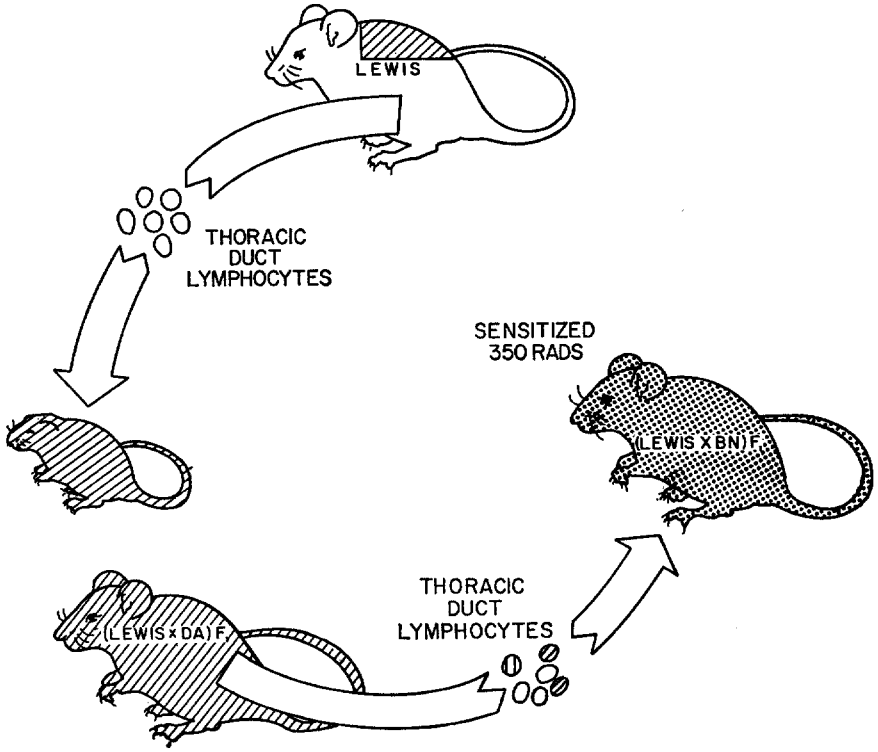

FIG. 3. Schematic representation of assay for immunologically competent parental strain lymphocytes in tolerant parental F_1 chimeras.

experiments, 25×10^6 thoracic duct lymphocytes from Lewis rats tolerant of (Lewis $\times$ DA)F_1 hybrid skin grafts were injected intravenously into newborn recipients syngeneic with the skin donor. The inoculated ainmals gained weight at approximately the same rate as normal, uninoculated littermates, and they showed no obvious evidence of "runt disease" (19). 8–15 wk after inoculation, the animals served as donors of lymphocytes for sensitized and X-irradiated adult (Lewis $\times$ BN)F_1 rats.

Table V indicates that 10^8 thoracic duct lymphocytes from parental lymphocyte–inoculated F_1 donors killed 1 of 10 secondary F_1 hosts. In contrast, an

equal number of lymphocytes from donors inoculated with normal Lewis bone marrow cells were lethal for 26 of 29 similar recipients.

Lymphocytes from Lewis rats tolerant of (Lewis × DA)F_1 skin are less effective than normal lymphocytes in causing a graft vs. host reaction in (Lewis × DA)F_1 rats. However, lymphocytes from such donors react with almost normal vigor against recipients belonging to the (Lewis × BN)F_1 hybrid cross (18). In the present experiments, newborn (Lewis × DA)F_1 rats were inoculated with approximately 25 times the dose of lymphocytes lethal for X-irradiated adult members of the (Lewis × BN)F_1 cross. Failure of thoracic duct lympho-

TABLE V

Effect of an Intravenous Injection of 10^8 (Lewis × DA)F_1 Hybrid Thoracic Duct Lymphocytes on (Lewis × BN)F_1 Hybrid Rats

Treatment of (Lewis × DA)F_1 donor		Fate of (Lewis × BN)F_1 recipient*		
Neonatal inoculum	Age when cannulated	No. inoculated	No. ill	No. killed
	wk			
Normal bone marrow‡	6–18	29	29	26
"Tolerant" lymphocytes§	8	3	0	0
	9	2	1	0
	15	5	2	1
Not inoculated	8–18	23	2	0

* Female rats 60–90 g body weight. The animals, sensitized to (Lewis × DA)F_1 tissues, were exposed to 350 rads of whole body X-irradiation 24 hr before injection.

‡ Male and female rats inoculated at birth with 5×10^7 normal Lewis bone marrow cells.

§ Male and female rats inoculated at birth with 25×10^6 thoracic duct lymphocytes from Lewis rats tolerant of (Lewis × DA)F_1 skin grafts.

cytes from the lymphocyte-inoculated F_1 donors to cause a graft vs. host reaction in the majority of secondary hosts is important for two reasons. First, it indicates that rat bone marrow is a potent source of potentially competent lymphocytes when compared with the peripheral lymphocyte pool. Second, it suggests that the potential immunological capability of bone marrow resides in cells normally resident in marrow and not in its content of circulating lymphocytes.

DISCUSSION

Adult rat bone marrow contains a small number of circulating immunologically competent small lymphocytes. In the current investigation, parental strain bone marrow cells were 50–100 times less effective than parental

strain thoracic duct lymphocytes in causing a graft vs. host reaction in X-irradiated, F_1 hybrid rats. The view that the weak immunological capability of bone marrow is invested solely in its content of itinerant small lymphocytes is based on experiments using marrow from lymphocyte-depleted donors. Large numbers of bone marrow cells from parental strain rats depleted of small lymphocytes by chronic lymph drainage had no obvious effect on irradiated F_1 hybrid recipients. In the rat, chronic lymph drainage causes a reduction of small lymphocytes in peripheral blood, thoracic duct lymph, and lymphoid tissue, and a nonspecific immunological deficiency (13, 15, 20). Failure of bone marrow cells from lymphocyte-depleted donors to cause a graft vs. host reaction in genetically susceptible recipients suggests that lymph drainage also depletes bone marrow of circulating, immunologically competent, small lymphocytes.

Small lymphocytes capable of initiating a lethal graft vs. host reaction can develop from cells in rat bone marrow. The present study indicates that, in adult F_1 rats injected intravenously at birth with parental strain bone marrow, the thoracic duct lymph contains cells having the immunological capability of the marrow donor. The fact that donor cells enter thoracic duct lymph suggests that they are *small* lymphocytes. In the rat, small lymphocytes are the only cells which circulate in large numbers from blood to thoracic duct lymph (12), and they alone have been implicated in graft vs. host reactions (1, 21).

Several lines of evidence support the view that immunologically competent small lymphocytes develop from myeloid precursors and not from circulating lymphocytes which become trapped in marrow. In F_1 rats inoculated with parental bone marrow, the immunological capability of cells in the peripheral pool of circulating small lymphocytes was greater than that originally present in the marrow inoculum. Thus, 5×10^7 normal Lewis bone marrow cells had the immunological capability of no more than 10^6 Lewis thoracic duct lymphocytes. Yet 6–18 wk after injection of 5×10^7 Lewis bone marrow cells into newborn F_1 rats, about the same immunological potential was embodied in 10^8 thoracic duct lymphocytes from the marrow recipient. The results imply that the recipient's circulating lymphocyte pool contained approximately 15×10^8 parental strain lymphocytes. This conservative estimate is based on two assumptions. First, the circulating pool in an adult rat contains 15×10^8 small lymphocytes (12). Second, the ability of Lewis lymphocytes to cause a graft vs. host reaction in (Lewis $\times$ BN)F_1 rats is unaffected by passage of the lymphocytes through a (Lewis $\times$ DA)F_1 host.

A second, more compelling argument against the possibility that immunologically competent small lymphocytes develop from circulating lymphocytes in marrow is provided by experiments using bone marrow from lymphocyte-depleted rats. Bone marrow cells from lymphocyte-depleted Lewis donors had no obvious effect on X-irradiated (Lewis $\times$ BN)F_1 hybrid recipients. However, lymphocyte-deficient bone marrow, like normal marrow, was capable of generating competent lymphocytes after injection into an intermediate F_1 host.

The view that the potential immunological capability of rat bone marrow resides in precursors of small lymphocytes and not in mature, immunologically competent cells receives some support from a study in which F_1 rats were inoculated with parental spleen cells (10). In the adult rat, the spleen is a potent source of immunologically competent cells as testified by the ability of normal spleen cells to cause graft vs. host reactions (19). However, Goldschneider and McGregor (10) found that a dose of Lewis spleen cells just smaller than that required to induce runt disease was unable to confer the donor's immunological capability on newborn (Lewis × DA)F_1 rats. Thoracic duct lymphocytes obtained from such animals in adult life failed to cause a graft vs. host reaction in sensitized and X-irradiated (Lewis × BN)F_1 hybrid recipients.

The present experiments, using lymphocytes from immunologically tolerant donors, virtually exclude the possibility that the potential immunological capability of rat bone marrow is invested in circulating small lymphocytes. In these experiments, newborn (Lewis × DA)F_1 rats were injected intravenously with thoracic duct lymphocytes from Lewis donors tolerant of (Lewis × DA)F_1 skin grafts. The number of lymphocytes inoculated was about 25 times the dose lethal for X-irradiated adult members of the (Lewis × BN)F_1 hybrid cross. However, 8–15 wk after inoculation, thoracic duct lymphocytes obtained from such animals had no obvious effect on the majority of (Lewis × BN)F_1 rats.

It could be objected that lymphocytes from tolerant rats fail to confer the donor's immunological capability on F_1 recipients because the donors are chimeric. Being chimeric, they contain two populations of lymphocytes: one is the animal's own, the other is derived from cells in the tolerance-inducing bone marrow inoculum. There is no information about the chimeric status of tolerant rats employed in the present experiments. However, two observations suggest that no more than a small portion of their circulating lymphocytes were derived from F_1 bone marrow. First, thoracic duct lymphocytes from Lewis rats rendered tolerant by injections of (Lewis × DA)F_1 bone marrow react with almost normal vigor against members of the (Lewis × BN)F_1 hybrid cross (18). Second, when the roles of bone marrow donor and recipient are reversed, as in the present experiments, only 1% of thoracic duct lymphocytes embody the immunological capability of the marrow donor.

It is not known why 3 of 10 recipients of lymphocytes from parental lymphocyte–inoculated F_1 donors developed homologous disease (Table V). Perhaps thoracic duct lymph contains a small number of competent lymphocyte precursors. The precursors could be large lymphocytes, immunologically "immature" small lymphocytes, or both. Another possibility is that small lymphocytes fully capable of reacting against BN tissue isoantigens join the intermediate host's circulating lymphocyte pool. Weeks later, these cells would be withdrawn from the animal's thoracic duct. The idea that homologous disease is caused by small lymphocytes originally present in the parental lymphocyte inoculum is con-

sistent with the long life-span of many rat small lymphocytes (22, 23) and their ability to circulate from blood to thoracic duct lymph (12, 24, 25).

Studies using chromosome markers have shown that bone marrow cells can colonize lymphoid tissue via the blood (4, 26). In lethally X-irradiated mice with an intact thymus or syngeneic thymus graft, injected bone marrow cells repopulate lymphoid tissue, the thymus included. Similarly, in nonirradiated mice joined in parabiotic union, cells (presumably from marrow) move from each animal into the thymus of its partner (26, 27). The results suggest that thymus receives immigrant cells from bone marrow and that these cells, or their progeny, are exported to spleen, lymph nodes, and Peyer's patches. However, the results do not reveal the immunological capability of the migrant cells. It is still debated whether cells from marrow generate competent lymphocytes while resident in thymus or whether lymphocytopoiesis in other tissues is controlled by a thymic humoral factor. A combination of the two is possible: thymus could provide a fertile *milieu* for development of potentially competent myeloid cells, and by a humoral mechanism it could regulate the development of competent lymphocytes in peripheral lymphoid organs (4). The fact that thoracic duct lymph is relatively deficient in competent lymphocyte precursors when compared with bone marrow and thymus (10) suggests that only mature immunologically competent lymphocytes join the circulating lymphocyte pool.

The present experiments indicate that rat bone marrow is a reservoir of competent lymphocyte precursors. However, no information is provided about the identity of the precursor cell type(s), the size of the precursor pool, or the anatomical location in which these cells or their progeny acquire the capacity to undertake an immunological response.

SUMMARY

Rat bone marrow is deficient in immunologically competent cells when compared with the peripheral pool of circulating small lymphocytes. However, circulating lymphocytes capable of initiating a lethal graft vs. host reaction can develop from myeloid precursors. In adult rats inoculated at birth with parental strain bone marrow cells, the thoracic duct lymph contains cells having the immunological capability of the marrow donor. The evidence suggests that immunologically competent lymphocytes derive from cells normally resident in bone marrow and probably not from precursors in the peripheral lymphocyte pool.

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