The Ablation of Sensitization to Bone Marrow Allografts[1] (36861)

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Many of the patients who might be considered as possible candidates for hematopoietic cell grafts will inevitably have received multiple blood transfusions (1–4). In such patients the immuno-suppressive therapy which must be exploited in order to allow transplanted allogeneic hematopoietic cells to become established is likely to be complicated. It has been demonstrated that transfusions of blood from donors which may be unrelated to both the recipient and the hematopoietic-cell donor may inhibit the subsequent survival of injected hematopoietic cells which would otherwise proliferate and become established in dogs (5–7) and in monkeys (8). Such inhibition may occur even after a single blood transfusion (5, 6). No methods for the abrogation of this inhibition, which is presumably due to acquired immunity to antigens carried by the injected cells, have been devised. The present investigation has therefore been undertaken in an attempt to do so, in the belief that with the introduction of fractionated hematopoietic-cell suspensions (9–12), satisfactory immuno-suppressive agent (13, 14) the refined methods for matching recipients with appropriate donors (15, 16), hematopoietic-cell transplantation may soon find a useful place in the therapy of hematopoietic insufficiency (17).

Mice which have rejected hematopoietic cells from an allogeneic donor, unlike mice which have not done so, cannot be protected against the lethal effects of a high dose of whole-body X irradiation by the injection of hematopoietic cells derived from donors of the allogeneic strain (18–21). The immunity acquired to allogeneic hematopoietic cells is thus not eradicated by a lethal dose of whole body X irradiation and in this respect resembles the immunity acquired during the rejection of a tumor allograft (22, 23). As the immunity induced by tumor allografts can be eradicated by anti-lymphocyte serum (22–24) the possibility suggests itself that the immunity induced by cells derived either from the blood or from the hematopoietic tissues of an allogeneic donor may be similarly eradicated.

Materials and Methods. CBA (Birmingham) mice of both sexes, aged 7 ± 1 weeks (supplied by OLAC Northern) have been used for this investigation, together with CSI/Ash mice of similiar age (supplied by Scientific Products Farm). CSI mice have previously been shown to reject allografts of a CBA mammary adenocarcinoma in less than 2 weeks (22, 23). Ten million bone marrow cells from CSI donors were administered by intra-peritoneal injection to CBA recipients which were thus sensitized to the iso-antigens of CSI bone marrow cells. The method used for the preparation of cell suspensions has been described in detail elsewhere (25). Sensitized CBA mice were divided into 8 groups, to which 3 groups of unsensitized CBA mice were added. Males and females were evenly distributed between these groups which were treated as follows:

1. Untreated control (unsensitized)
2. Irradiated (unsensitized)
3. Sensitized, treated with normal rabbit serum (NRS) and irradiated
4. Irradiated and treated with bone marrow cells (unsensitized)
5. Sensitized, irradiated and treated with bone marrow cells
6. Sensitized, treated with isotonic saline, irradiated and treated with bone marrow cells

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TABLE I. The Nucleated-Cell Content of the Medullary Cavity of the Femoral Diaphysis in the Eleven Groups of Mice Which Have Been Studied.

Group number	Sensitized	Treatment	Irradiated	Test graft dose $\times 10^{-7}$	Femoral marrow cellularity $\times 10^{-6}$ mean $\pm$ SE	No. of animals
1	—	Control	—	—	10.20 $\pm$ 0.40	10
2	—	—	+	—	0.84 $\pm$ 0.08	7
3	+	NRS	+	—	0.64 $\pm$ 0.09	9
4	—	—	+	1	8.23 $\pm$ 0.36	13
5	+	—	+	1	1.80 $\pm$ 0.33	14
6	+	Saline	+	1	1.25 $\pm$ 0.31	5
7	+	NRS 1	+	1	4.52 $\pm$ 0.78	9
8	+	NRS 2	+	1.4	6.80 $\pm$ 1.44	10
9	+	NRS 3	+	1.4	5.20 $\pm$ 0.88	10
10	+	ALS 1	+	1	4.38 $\pm$ 1.71	5
11	+	ALS 2	+	1	5.41 $\pm$ 0.83	10
						102

7. } Sensitized, treated with various batches
8. } of NRS, irradiated and treated with
9. } bone marrow cells
10. } Sensitized, treated with various batches
11. } of antilymphocyte serum (ALS), irradiated and treated with bone marrow cells

Rabbit-anti-mouse lymphocyte serum has been prepared using two injections of mouse thymocytes, in New Zealand white rabbits (22). The serum was heat inactivated at 56° for 1 hr and stored at —20°. Normal rabbit serum was collected and treated similarly.

The immuno-suppressive potency of antilymphocyte sera was assayed by measuring the ability of different batches to suppress the rejection of tumor allografts (26).

Saline, NRS and ALS were administered by intraperitoneal injection in two 1 ml pulses on the 8th and 10th days after the sensitizing injection of bone marrow cells.

On the 18th day after sensitization mice in groups 2–11 were exposed to a single dose of whole-body X irradiation (820 rads, 300 kVp, 5 mA, 0.5 mm Cu + 5 mm Al filtration, dose rate 64 rads/min) and those in groups 4–11 received 10^7 or 1.4×10^7 bone marrow cells from CSI donors by intravenous injection shortly afterwards (25). Ten days later, on the 28th day after sensitization, mice were anesthetized by exposure to ether vapor and samples of femoral bone marrow were collected. The nucleated-cell content of the femoral diaphysis was measured using a Coulter Counter Model B. Smears prepared from suspensions of femoral bone marrow cells in mouse serum were stained using a variant of the Jenner–Giemsa technique, which has been found to be especially satisfactory for murine hematopoietic cells (25).

Results. The results are summarized in Table I. The cellularity of the femoral bone marrow which has been used to assess repopulation of the medullary cavity of the femoral diaphysis has previously been shown to be a useful index of the proliferation of transplanted hematopoietic cells in lethally irradiated mice (27).

Ten days after the administration of 820 rads of whole-body X irradiation, the nucleated-cell content of the medullary cavity of the femoral diaphysis is reduced from 10^7 (group 1) to less than 10^6 (group 2). Following an intravenous injection of 1×10^7 bone marrow cells the cellularity of the femoral bone marrow is almost restored within 10 days (group 4). In animals which have previously been sensitized to the iso-antigens of the donor bone marrow cells, however, restoration does not occur (groups 5 and 6. Unsensitized: sensitized $p << 0.001$). Following treatment with NRS the nucleated-cell con-

tent of the medullary cavity of the femoral diaphysis is partially restored (groups 7, 8 and 9). NRS does not modify the radiosensitivity of the bone marrow in sensitized mice (group 3). In animals treated with ALS also partial restoration of the cellularity of the femoral bone marrow has been observed (groups 10 and 11).

The distribution of nucleated cells between the principal morphological categories is similar in the cell populations which are established in the medullary cavity of the femur in all 6 of the groups in which restoration or partial restoration of the cellularity of the bone marrow has been observed (groups 4 and 7–11).

Discussion. Cells derived from allogeneic bone marrow are incapable of repopulating the medullary cavity of the femur in lethally irradiated recipients which have previously been sensitized to the iso-antigens of the donor cells. The present investigation was undertaken in the hope that ALS might eradicate this acquired immunity of the recipient. While it has been demonstrated that ALS is capable of doing so, however, NRS also possesses this property. In the instance studied, therefore, abrogation of acquired immunity cannot be attributed to any property of ALS which is not shared with NRS. Thus ALS and NRS may both exert comparable immunosuppressive effects or, alternatively, both may contain antigens which compete with the iso-antigens of the allogeneic bone marrow cells. The mechanism by which acquired immunity has been abrogated is currently being investigated. Meanwhile it seemed desirable to record the feasibility of devising a regime which will allow allogeneic bone marrow cells to proliferate in a previously sensitized recipient, in view of the potential clinical importance of such a regime and because no previous investigators have suggested the possibility of doing so.

Summary. Mice which have been immunized against the isoantigens of allogenic bone marrow cells reject bone marrow cells from donors of the allogeneic strain that are injected after the administration of a lethal dose of whole-body X irradiation. Repopulation of the damaged hematopoietic tissues is therefore prevented. It has now been demonstrated that treatment with anti-lymphocyte serum or normal rabbit serum abrogates such immunity, as allogeneic bone marrow cells repopulate the medullary cavity of the femur in sensitized recipients treated with anti-lymphocyte serum or normal rabbit serum before being exposed to a lethal dose of whole-body X irradiation.

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