THE COLONISATION OF IRRADIATED TISSUE BY TRANSPLANTED SPLEEN CELLS

N. A. MITCHISON

From the Medical Research Council Radiobiological Unit, Harwell, and the Zoology Department, University of Edinburgh

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SPLEEN cells transferred from normal animals have been shown to counteract the lethal effects of ionising radiation in mice, by Jacobson, Simmons, Marks, Gaston, Robson, and Eldredge (1951). Jacobson (1952) postulated that these cells act on the host tissue through a humoral agent. This hypothesis is supported by the evidence of Hilfinger, Ferguson, and Riemenschneider (1953), and Cole, Fishler, and Bond (1953), that cell-free preparations of tissue are also able to counteract radiation injury. On the other hand, Barnes and Loutit (1954*a*, *b*) argued that the irradiated host tissue is repopulated by the transplanted cells.

The purpose of the present paper is to trace the fate of normal spleen cells injected into irradiated mice. In one series of experiments the injected cells were identified in the hosts by their iso-antigens, the donors and hosts being taken from two inbred strains of distinct iso-antigenic type. The method of identification depends on the ability of the iso-antigens in tissue cells to elicit transplantation immunity in a foreign host. At intervals after irradiation and injection, tissues of the host were transplanted into fresh hosts of the same strain. When the injected spleen cells or their descendants were still present in these tissues, they elicited in their fresh hosts a heightened resistance to grafts of their own antigenic type. For testing this graft resistance, an A-strain tumour (Sarcoma 1) was employed of which the growth in normal and immunised hosts had already been studied (Mitchison, 1955). In foreign hosts the tumour normally attains considerable size before regressing, while in hosts which have been previously immunised the growth is slight. This difference was used as the criterion of immunisation.

In a second series, injections were made of spleen cells which had been immunologically activated by intravenous injection of a bacterial antigen into the donors. The production of antibody by the cells in their hosts then served as a method of identification.

METHODS

Animals

The mice used in this study were of the A and CBA standard inbred strains.

Radiation

Five mice at a time were irradiated in aluminium cages, a uniform ($\pm$ 3 per cent) X-ray beam being directed through the floor of the cage. The radiation was produced by a Westinghouse radiotherapeutic X-ray machine operated at 240 kV and 15 mA and had a Half Value Layer of 1·2 mm. Cu. The dose rate 1·5 cm. above the sawdust-covered floor was 43 R per min. Those mice which received spleen cells identified by iso-antigens were given

950 R, a dose shown to be 100 per cent lethal to control mice (Barnes and Loutit, 1954c). Those mice which received immunologically activated spleen cells, and which were subsequently placed under the stress of repeated bleedings, were given a dose of 500 R.

Spleen cell transfer

Spleen cells to be identified by their iso-antigens were obtained from 8-day-old A mice. The spleens were mechanically minced by a rotating blade which reduced them to a suspension of individual cells and small cell clumps. The suspension was injected intravenously into irradiated and control CBA mice immediately after irradiation. Each host received $\frac{2}{5}$ of a spleen, approximately 15×10^6 cells.

Spleen cells to be identified by their production of antibody were obtained from adult donors. These spleens were also mechanically minced, and were injected into the peritoneum of irradiated and control mice at a dosage of one spleen per host.

Test for iso-antigens

Tissues to be tested for the presence of A iso-antigens were weighed, mechanically minced, and injected into the peritoneal cavity of CBA mice. Except in the case of peritoneal exudates, the tissues from each individual were tested separately, the quantities of tissue transferred being as follows: spleen, the whole organ; lymph nodes, the pooled axillary, brachial, inguinal nodes; liver, approximately 100 mg.; blood, 0·4 ml.; peritoneal exudate, containing 50–60 per cent macrophages and the rest lymphocytes, obtained from the peritoneal cavity 2 days after injection of 1 ml. paraffin (Mitchison, 1954).

After an interval of 8–12 days, the CBA hosts were inoculated subcutaneously with a standard test dose of 1×10^6 cells of the A strain sarcoma, Sarcoma 1. For the present study, the tumour was maintained in the ascites form by serial transfer in A mice. The tumours grown from the test inocula were excised and weighed after 8 days. Those mice which had been previously exposed to A iso-antigens, by the first injection of tissue, were expected to show smaller growths of tumour. Control groups of normal and well immunised CBA mice were included in each series inoculated with the tumour.

Transfer of antibody production

Spleen-cell donors were immunised by one or more intravenous injections of 0·2 ml. of a commercial preparation of *Salm. typhi* (H) antigen (Burroughs Wellcome standard agglutinable suspension). Separate samples of blood for agglutinin titration were obtained from each mouse by bleeding from the ventral tail artery.

RESULTS

Changes in spleen weight after irradiation

CBA mice which received a dose of 950 R of X-rays showed a rapid decline in the weight of the spleen. Without injection of spleen cells the irradiated mice died after $6{\cdot}3 \pm 0{\cdot}9$ days. Fig. 1 shows that injection of spleen cells brought about a gradual recovery in the weight of the host spleen, starting after 4–7 days. The possibility that the transplanted cells may have produced this recovery by colonising the host spleen is the subject of further investigation.

Sensitivity of the test for iso-antigens

Table I shows the growth after 8 days of the A tumour in untreated CBA mice, and in control CBA mice in which a state of immunity against A tissue had been previously induced. The control immunity was induced at least 15 days before the test inoculations of tumour, by subcutaneous inoculation of the same tumour, followed by growth and regression. Batches of normal and immunised mice were inoculated from the same pool of tumour cells, together with various experimental

groups of mice ; each inoculum is numbered in the Tables, and some variation between inocula is apparent. The state of immunity is shown by the smaller growths of tumour among the mice which were previously inoculated. The mean weight of tumour, and its standard deviation, were calculated on a logarithmic scale for each batch of normal and immunised mice. Each mouse whose state of immunity was under test could then be assigned to either group.

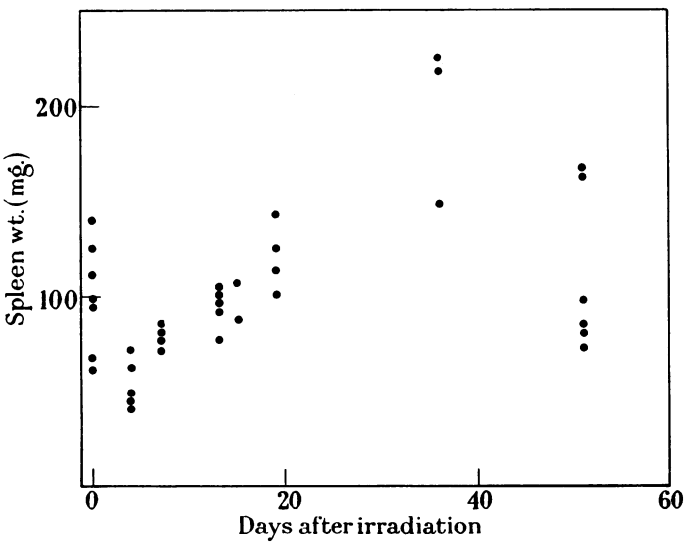

FIG. 1.—Spleen weight in irradiated mice after transplantation of spleen cells. Figs. 2–6 show titrations of individual sera, with points spaced separately for clarity. Each curve represents a single mouse.

The quantity of tissue from adult A mice needed to induce immunity is shown in Table II. A 1/125th fraction of a spleen (10^6 cells), or approximately one-tenth as many cells from a peritoneal exudate, is sufficient to produce a detectable state of immunity.

TABLE I.—*Growth of A Tumour in Normal and in Previously Inoculated CBA Mice*

Tumour inoculation no.	Weight (mg.) of tumour implants after 8 days.
	Normal CBA hosts.
1 . .	82, 146, 176, 212, 360
2 . .	104, 124, 128, 206, 218, 230
3 . .	48, 63, 66, 72, 126, 157, 197, 210
4 . .	332, 380, 442, 504, 637, 652
5 . .	192, 228, 232, 274, 294, 352, 450, 877
	CBA hosts previously inoculated with A tumour.
1 . .	8, 19, 25, 39, 42
2 . .	2, 2, 3, 4, 4, 9
3 . .	2, 2, 6, 10
4 . .	16, 17, 24
5 . .	7, 10, 12, 18

TABLE II.—*Estimation of the Quantity of A Tissue needed to induce Transplantation Immunity in CBA Mice*

(Weight (mg.) of tumour implants after 8 days.)

Tumour inoculation no.	Undiluted cell count.	Hosts previously injected with dilutions of cell suspension from one A spleen: 1/2.	1/5.	1/25.	1/125.	1/625.	1/3125.
1	151×10^6	—	8†	3†	3†	94*	—
3	120×10^6	3†	4†	2†	1†	—	—
5	178×10^6	—	12†	19†	22†	136	223
5	81×10^6	—	—	19†	96†	284	533
		Hosts previously injected with dilutions of A peritoneal exudate:					
3	$2{\cdot}8 \times 10^6$	2†	2†	27*	—	—	—
		9†	—	—	—	—	—
5	$4{\cdot}3 \times 10^6$	33*	9†	19†	90*	196	—
		65*	202	108*	352	772	—

* Significantly less than controls in normal hosts.

† Significantly less than controls in normal hosts, and not significantly more than controls in immunised hosts.

Fate of iso-antigens in irradiated and normal hosts

Table III shows the results of tests for the presence of A strain iso-antigens in tissues of the irradiated CBA mice which received spleen cells from A donors. Tests of various tissues obtained from single irradiated animals are shown on each line. No A strain iso-antigens were detected in liver or blood. Some variation between individuals is apparent in respect of the spleen, lymph nodes, and peritoneal exudates; but over the full duration of the investigation, from 4 until 51 days after irradiation, the iso-antigens of the transplanted cells could be detected in these sites in at least some animals.

The results of similar tests, carried out on the tissues of normal CBA mice which received spleen cells from A donors, are shown in Table IV. The iso-antigens of the transplanted cells were detected in the spleen after 4 and 7 days, but not later. No indications were found of their presence in the lymph nodes or blood.

Quantitative tests carried out on the spleens of irradiated mice did not give fully reliable results. But indications were given that a large fraction of the spleen may be of the donor antigenic type after irradiation and injection of spleen cells.

Antibody production in irradiated and normal mice receiving immunologically activated spleen cells

Preliminary experiments, shown in Fig. 2, established that *Salm. typhi* (H) antigen stimulated adequate production of antibody, and that irradiation is an effective agent in impairing production of this antibody.

Immunologically activated cells from the spleens of immunised mice were then transferred into irradiated and into normal host mice. In one series, shown in Fig. 3, the donors received a single injection of antigen 10 days before the transfer. In another, shown in Fig. 4, the transfer was carried out 4 days after a second injection of antigen into the donors. Both series show greater production of antibody in the irradiated than in the normal hosts. Donors and hosts were both

TABLE III.—*Detection of A Iso-antigens in Irradiated CBA Mice, After Transplantation of Spleen Cells.*

Tumour inoculation no.	Interval after irradiation (days).	Tissue transferred from irradiated mice ; weight (mg.) of subsequent tumour implants after 8 days.				
		Spleen.	Lymph nodes.	Peritoneal exudate.	Blood.	Liver.
5	4	14†	388	—	—	—
		6†	42*	—	—	—
		—	116*	—	—	—
		—	62*	—	—	—
		—	40*	—	—	—
4	7	43*	198*	—	—	—
		219	104*	—	—	—
		135	268	—	—	—
		284	124*	—	—	—
		21†	270	—	—	—
5	13	—	30*	—	—	—
		—	16†	—	—	—
		—	144	—	—	—
		—	64*	—	—	—
		—	12†	—	—	—
1	14	38†	54†	—	277	72
		22†	168	—	275	135
		369	10†	—	93	105
		40†	60	—	94	306
		57†	17†	—	205	102
2	16	—	—	3†	—	—
		—	—	1†	—	—
2	19	40*	—	—	—	—
		21*	—	—	—	—
		4†	—	424	—	—
		61*	—	60*	—	—
		98	—	151	—	—
3	36	—	—	7†	—	—
		—	—	3†	—	—
4	51	32*	21†	—	—	—
		19†	22†	28†	—	—
		356	20†	—	—	—
		18†	12†	—	—	—
		68*	16†	38*	—	—
		47*	160	—	—	—

* Significantly less than controls in normal hosts.
† Significantly less than controls in normal hosts, and not significantly more than controls in immunised hosts.

of the A strain in the first series ; in the second strain the donors were all of the A strain, so that a comparison could be made of homologous transfer with transfer into a foreign strain. The relationship of donor and host does not appear to affect the outcome of the transfer under these conditions.

Impaired production of antibody in a foreign host should nevertheless be demonstrable, if the transplanted cells were again transplanted from A donors into

TABLE IV.—*Detection of A Iso-antigens in Normal CBA Mice, After Transplantation of Spleen Cells.*

Tumour inoculation no.	Interval after irradiation (days).	Tissue transferred from irradiated mice; wt. (mg.) of subsequent tumour implants after 8 days.		
		Spleen.	Lymph nodes.	Blood.
4	4	130*	318	—
		358	330	—
		121*	409	—
		397	990	—
		122*	559	—
5	7	422	—	—
		51*	—	—
		652	—	—
		326	—	—
		588	—	—
1	14	194	—	269
		374	—	114
		112	—	329
		178	—	100
		164	—	166
5	53	1156	—	—
		798	—	—
		566	—	—
		172	—	—
		236	—	—

* Significantly less than controls in normal hosts.

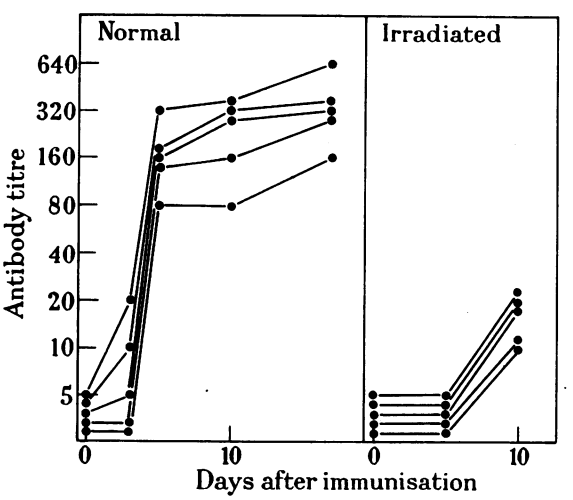

FIG. 2.—Antibody production in A mice after injection of *Salm. typhi* (H).

normal, and into irradiated CBA hosts, 4 days after a second injection of antigen. Half the normal, and half the irradiated hosts, had been previously immunised against A strain tissue by intravenous injection of 25×10^6 A spleen cells. Fig.

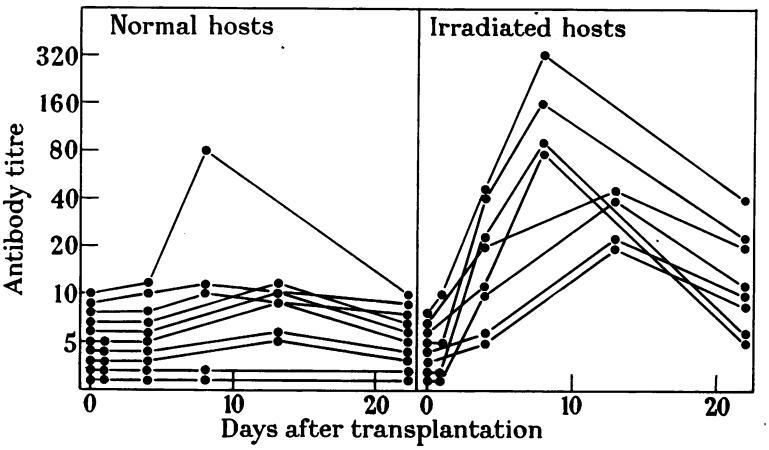

FIG. 3.—Antibody titres in hosts after transplantation of spleen cells from primarily immunised donors.

5 shows that production of antibody was impaired in the previously immunised hosts.

DISCUSSION

It has been shown that the iso-antigens of spleen cells which have been transplanted into irradiated mice are detectable in some of their hosts for at least 51 days, long after they have disappeared from normal hosts. This prolonged survival is evidence for the colonisation of the organs of the host by the transplanted cells; on this hypothesis, the host may properly be called a *radiation-chimaera*. An alternative hypothesis is that the iso-antigens of the transplanted cells become incorporated into the cells of the host. That this is the normal incorporation of foreign antigens into the tissues of the host is unlikely, in view of the large quantity of iso-antigen which was detected in some experiments, and the discrepancy between the normal and irradiated hosts. Cole *et al.* (1953) have discussed the possibility that material may be transferred to radiation-injured tissue analogously to bacterial transduction. This hypothesis is *prima facie* unlikely, but is not excluded by the present experiments.

It has also been shown that when spleen cells have been exposed to antigen and then transplanted, production of antibody is more active in hosts which have been previously irradiated. The present results with mice serve to confirm and extend the findings of Harris and Harris (1954) with rabbits. A source of confusion in the interpretation of this experiment is that antigen may be transferred together with the transferred spleen cells. But since irradiation has been shown to impair antibody production, immunological activation of host tissue cannot account for the discrepancy between normal and irradiated hosts. The enhanced production of antibody in the irradiated hosts may reasonably be explained as a result of multiplication of the transplanted cells.

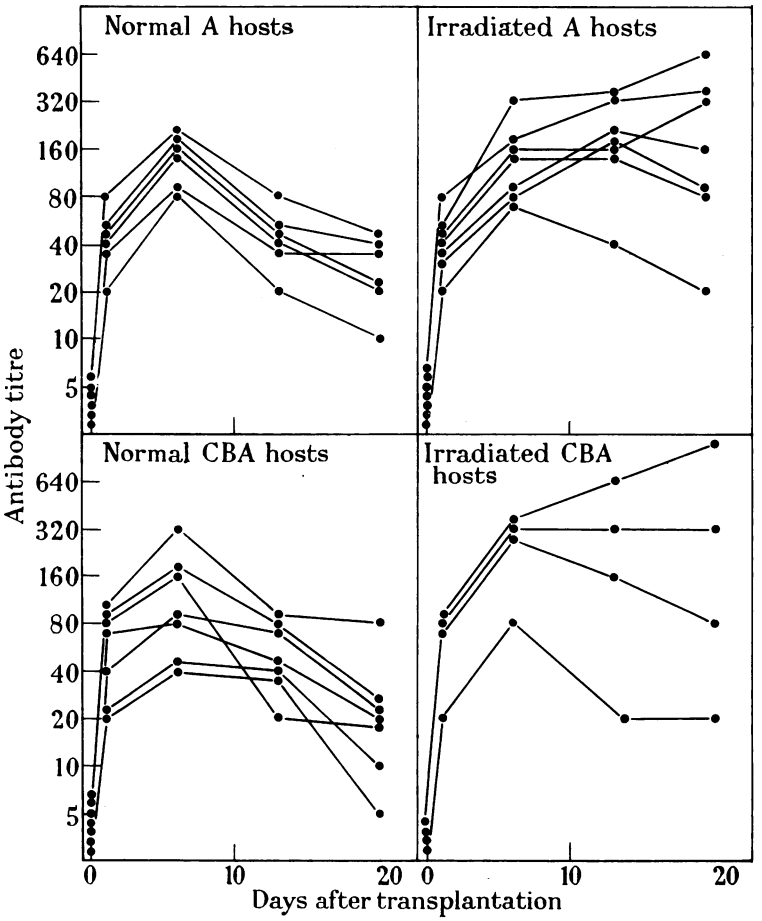

FIG. 4.—Antibody titres in hosts, after transplantation of spleen cells from secondarily immunised donors. Donors and hosts of the same strain (A) compared with donors and hosts of different strains (A and CBA).

The transferred iso-antigens were found only in the lymph nodes and spleen of the hosts. The transplanted cells therefore appear to have localised strictly in their homologous tissue. Similarly, Danchakoff (1918) and Ebert (1951) have shown that the embryonic spleen enlarges after grafting adult chicken spleen on the chorio-allantois.

SUMMARY

Spleen cells identified by iso-antigens were transplanted into irradiated mice. The iso-antigens were detected in the host lymph nodes and spleen up to 51 days after transplantation, by their ability to induce transplantation immunity.

Irradiation of the host enhanced the production of antibody by spleen cells transplanted from immunised donors.

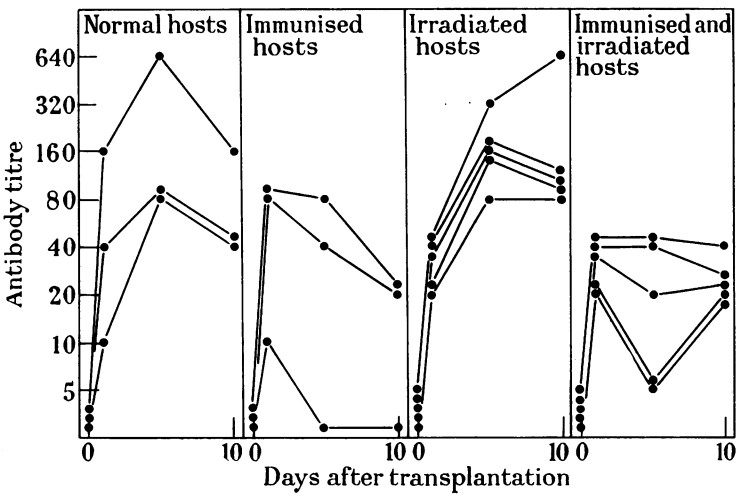

FIG. 5.—Antibody titres in hosts, after transplantation of spleen cells from secondarily immunised A donors. Normal CBA hosts compared with CBA hosts previously immunised against A tissue.

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