IMMUNOGENETIC PROBLEMS OF TISSUE TRANSPLANTATION

by

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Introduction

In 1903, HEINECKE first demonstrated that the principal cause of death after whole body irradiation of animals, is gross injury to the haematopoietic tissues. A few years later, BENJAMIN and SLUKA (1908) found that the immune response of an animal against foreign tissue grafts is suppressed by X-rays. These were important observations at that time. Since then very much more has been learned about the effects of radiation on living organisms. Research into the cause and remedy of radiation injuries have been particularly intensified in recent years, as a result of the discovery of nuclear fission and its application in the development of new weapons, in industry and in medicine, consequently increasing the danger of radiation hazards to man. One of the most spectacular therapeutic developments was the injection of bone marrow cells into heavily irradiated organisms to ameliorate the effect of injury to the haematopoietic system. The present paper is concerned with some of the immunological problems which arose as a result of such replacement therapy.

Bone marrow injury and recovery, in the irradiated organisms

Irradiation, given to the whole body of mice, produces damage to the blood forming tissues. The seriousness of such an injury is determined by the dose of radiation. The higher the radiation dose, the greater is the injury, which very often cannot be repaired by the organism's own resources and results in death. The dose which kills nearly all the irradiated mice or other animals within 30 days is referred to as the lethal dose (LD_{99}). Intravenous injection of haematopoietic cells (bone marrow, foetal liver or spleen) from a non-irradiated animal (called „the donor") can restore haematopoietic function in the irradiated animal (called „the recipient or host") by recolonising the haematopoietic organs and multiplying at these sites. Recovery from radiation injury is completed without any immunological complication when the recipient and bone marrow donor are mice of the same strains, i. e. genetically identical (isogenic).

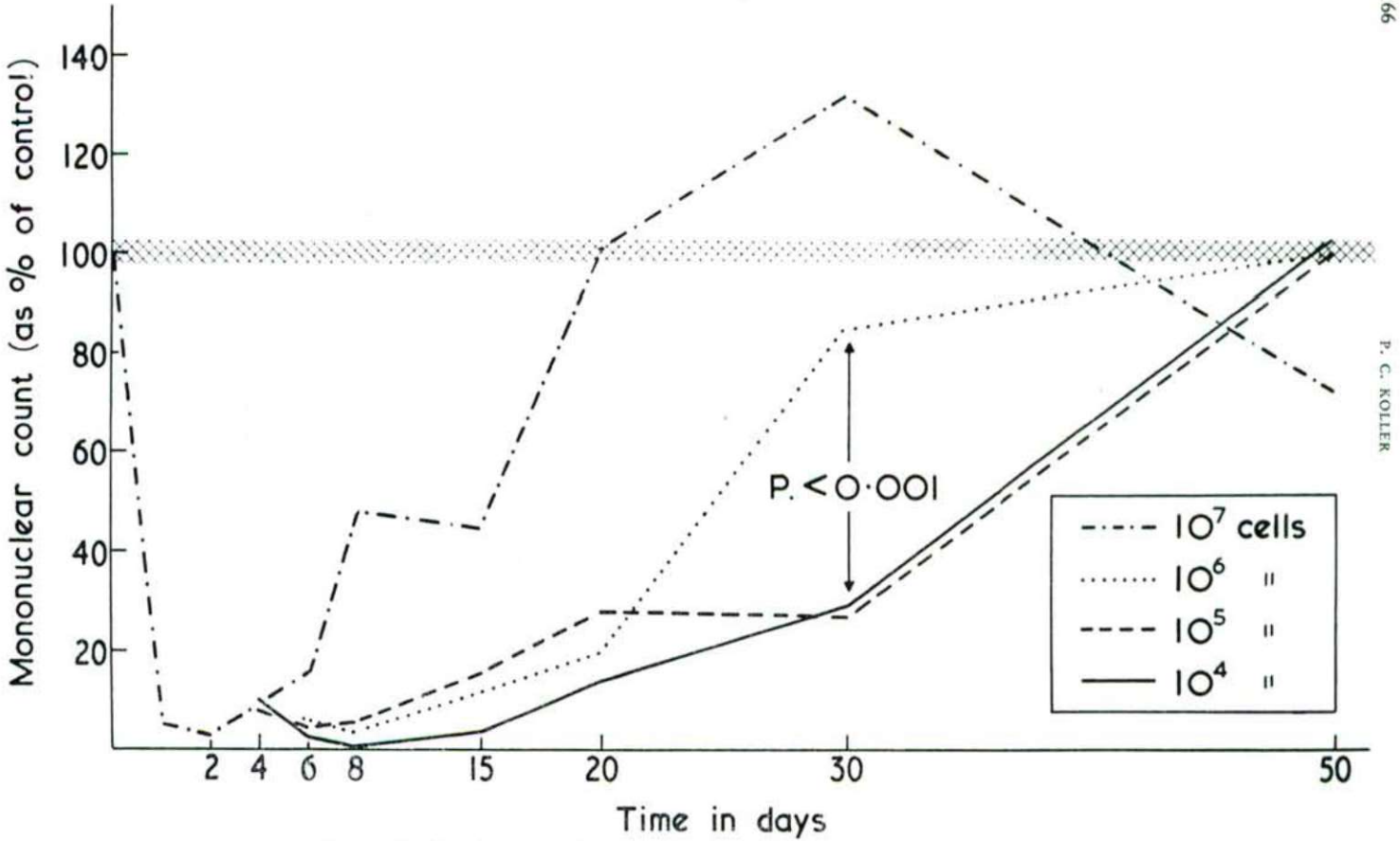

Figure 1 Graph showing the rate of recovery of mononuclear cells in Balb/c mice irradiated with 700r (LD_{99}) and given varying number of bone marrow cells.

The rate of recovery from radiation injury is influenced by the number of haematopoietic cells injected as therapy. By analysing the peripheral blood of the recipient, the rate of recovery can be followed. Figure 1 illustrates the effect of donor cells on the mononuclear cells in the blood of irradiated mice which received 700 r (LD_{99}). It can be seen that after the injection of 10^7 cells, the mononuclear cell count began to rise on the 6th day and reached the normal level by the 20th day. However, when 10^5 or 10^4 donor cells were injected, the mononuclear count remained well below the normal level at the 30th day.

Radiation effect on the immune response

Our experiments in which foreign tumours were implanted into irradiated mice confirmed the early results of BENJAMIN and SLUKA (1908) and of MURPHY (1914) that the natural resistance of an adult animal to heteroplastic tissue grafts can be suppressed by X-rays. We also demonstrated that the duration of suppression depends on the radiation dose. This observation was made by challenging the irradiated animal with foreign tissue at different intervals after radiation. Figure 2 illustrates the method employed and the results obtained. In these experiments the irradiated Balb/c mice were challenged at 3, 5, 10, 15 and 20 days after irradiated with Sarcoma 1 (Sa—1), a foreign tumour which arose in another strain (A.) The data show that at about 15 days after 300r, a sub-lethal dose of irradiation, the immune mechanism of the animal recovered. The arrest of tumour growth and its rejection indicate that antibodies are produced againts the foreign tumour antigens i. e. the lymphoid system is functioning normally.

Similarly in mice, given a lethal dose of irradiation and also therapeutically injected with bone marrow cells from mice of the same strain as the recipient, the immune response recovers after some delay. By using the same methods as were followed above, in which sub-lethal radiation was given, it was found that the immunological reactivity of the heavily irradiated animal is fully operative again at 25 days (Figure 3).

Restoration of immunological function by donor cells

The respective role of the recipient host and donor components in the immunological response of the lethally irradiated and bone marrow treated mice could not be definitely ascertained in the experiments described above because the irradiated host and marrow donor were genetically identical (isogenic). Such a study can only be made when the host and donor animals are genetically different. Organisms housing mixtures of genetically different cells or tissues are referred to as CHIMAERAS. Thus mice in which the haematopoietic tissues are destroyed by radiation and replaced from a „foreign" donor, can be rightly called *„radiation chimaeras"*.

The part played by the donor component in the immunological function of *chimaeras* can be revealed by transplantation of skin or tumour grafts of different origin. Such an experiment is to be described. Mice of CBA strain were irradiated with a lethal dose of X-ray and given bone marrow cell sus-

pensions prepared from the *femur* of mice of Balb/c strain. Five days after irradiation and marrow therapy, a number of the *chimaeras* were grafted with skins from CBA (host), and with Balb/c (donor) strains. It was found that

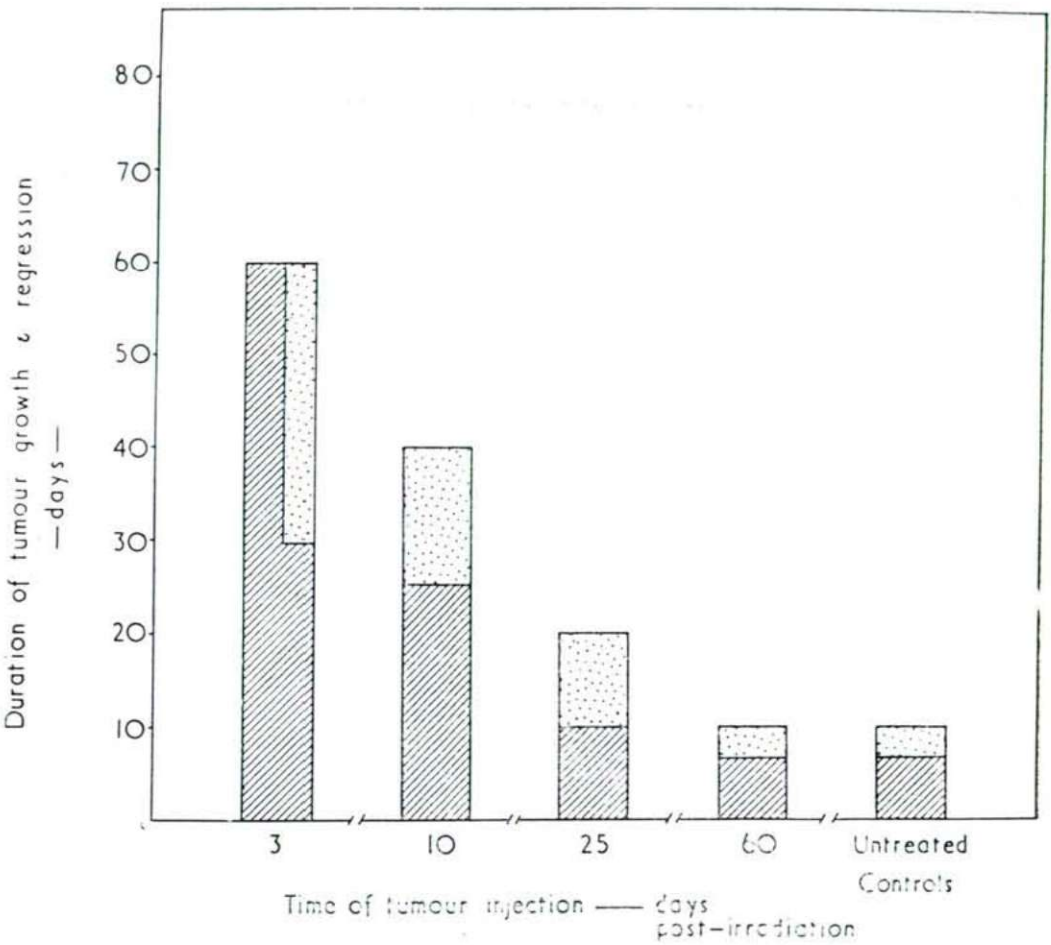

Figure 2 Histogram showing the growth of foreign tumour grafts inoculated at varying times after 300r total body radiation. (The striped areas indicate progressive growth; the shaded areas indicate progressive rejection.)

nearly all the *chimaeras* rejected the host type skin and kept the skingraft of the donor strain. When skin grafting was carried out 50 days after irradiation and marrow therapy, the result was nearly the same as can be seen in Table I. These findings show that the restoration of the immune response in the *chimaeras* was achieved by the cells introduced into the irradiated host with the donor inoculum.

By using other strain combinations and testing their immunological status by the above method, it was found again that the tissues of the donor were accepted in the majority of cases, while those of the host were rejected. This

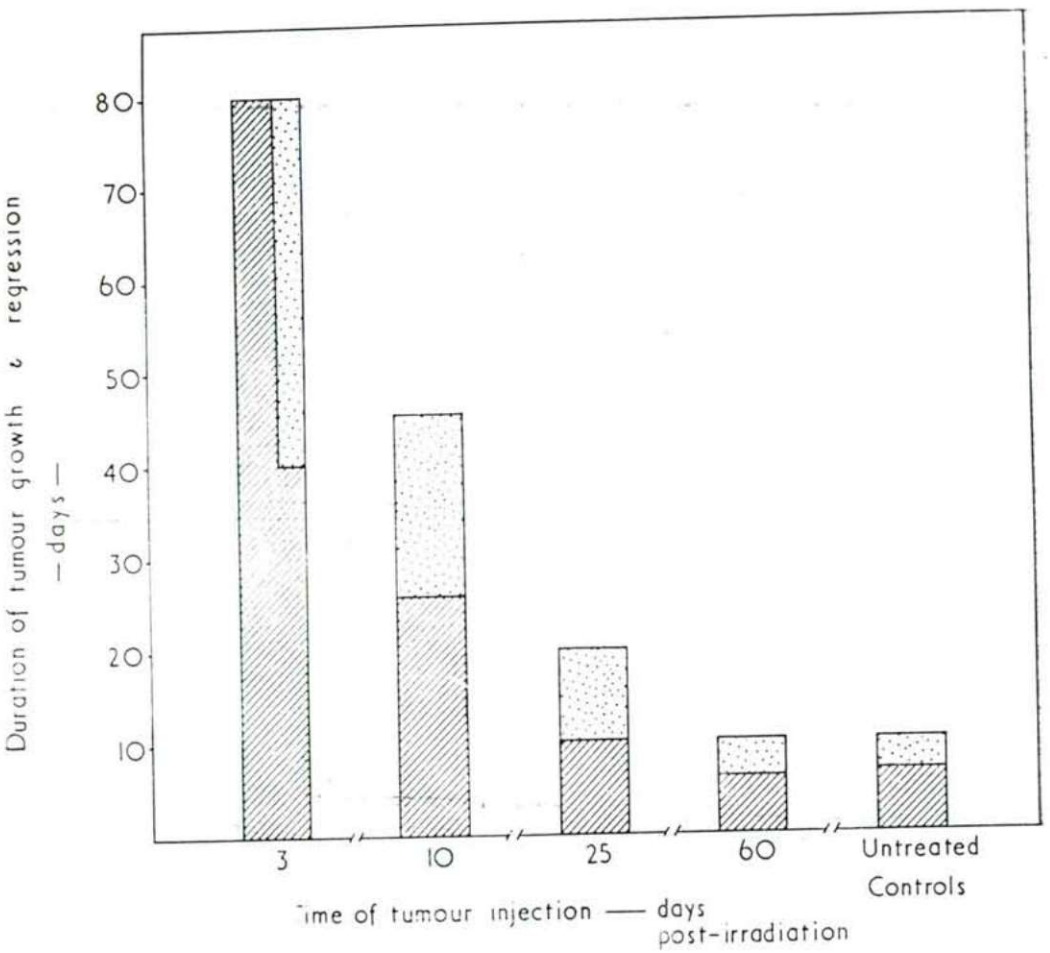

Figure 3 Histogram showing the recovery of immune response in mice which received 850r (LD_{99}) irradiated and given bone marrow. Mice were grafted with foreign tumour at varying intervals after irradiation, and marrow therapy. Note the similarity of the 'spontaneous' (Fig. 2) and 'assisted' restoration of immune response.

behaviour indicates that in such animals the immune reaction is directed against the antigens of the recipient. The anti-host reaction results in a chronic pathological condition, described as *„the wasting syndrome"* or *„secondary disease"*; mice suffering from this condition, lose weight, their fur becomes ruffled, and the skin displays *dermatitis*. *Diarrhoea* of varying degrees of seve-

TABLE I

The response of radiation mouse chimaeras of CBA (host) and Balb c (donor) constitution to skin grafts of host and donor origin

Days between irradiation and grafting	Marrow donor	Number of chimaeras	Skin grafts Accepted/Total number	
			CBA (host)	Balb/c (donor)
5	Balb/c	7*	0/7	7/7
50	Balb/c	24	1**/4	15/20

* Double grafted
** Graft small without fur growing.

TABLE II

Immunological response of chimaeras to tumour grafts

Chimaera		Tumour challenge			
Constitution Host/Donor	Age in days	Type	No. of takes	No. of chimaeras challenged	Type of reaction to graft
A/Balb/c	50	Sa-1 C+leuk. BP8		3/3 0/3 0/4	Host
C57BL/Balb/c	52—69	EL4 C+leuk. BP8		4/4 0/9 0/6	Host
CBA Balb/c	50	BP8 C+leuk. EL4		5/5 5/5 11/14	Host Donor Neutral
A/CBA	51	Sa-1 BP8 EL4		4/4 4/4 8/10	Host Donor Neutral
CBA/A	57—64	BP8 Sa-1 EL4		2/3 5/5 1/9	„Ill-defined"

Tumour specificity: BP8 CBA strain
Sa-1 A strain
C+leuk: Balb c strain
EL4 C57BL strain

rity is usually present and there is a generalised atrophy of the lymph nodes. It is now accepted that wasting syndrome, which usually causes the death of the animals between 30 and 50 days, in due to the immunological reaction of the foreign donor tissue against the recipient host, whose own immune mechanism has been destroyed by the irradiation (cf. KOLLER, DAVIES & DOACK 1961).

Radiation mouse *chimaeras,* however, have been observed in experiments which had survived or completely escaped the „wasting disease". These animals are of special interest because their survival suggests the successful coexistence of host and donor tissues. When the immunological behaviour of such *chimaeras* were tested by skin or tumour grafts, it was found that they displayed variable immunological responses. The results are given in Table II. Three patterns of behaviour have been observed: (i) *chimaeras* exhibiting the host's immunological characteristics, (ii) *chimaeras* with mixed and (iii) *chimaeras* with predominantly donor characteristics. The data strongly suggests that in these particular radiation *chimaeras,* the lymphoid component of the donor tissue has been altered.

Immunological status of chimaeric state

In order to obtain further information about the nature of the change which must have taken place in the radiation *chimaeras,* experiments were designed in which bone marrow of 50-day-old *chimaeras* was transferred into newly irradiated mice of the host or donor strains with a view to analysing the immunological behaviour of the new hosts.

Chimaeras of C57BL/Balb/c (host/donor) constitution were made which will be referred to as PRIMARY CHIMAERAS. Most of these *chimaeras* succumb to the chronic wasting syndrome. From the survivors of primary *chimaeras,* the bone marrow was transplanted into newly irradiated C57BL and Balb/c hosts, to give SECONDARY CHIMAERAS. A third transfer, 50 days later, from the 'se-

TABLE III

Survival of primary, secondary and tertiary chimaeras

Primary donor marrow	Hosts			Number of chimaeras	Survivors to 50 days (per cent)
	Primary	Secondary	Tertiary		
Balb/c	Balb/c	—	—	105	96
	C57BL	—	—	216	23
	C57BL	C57BL	—	40	57
	C57BL	Balb/c	—	35	28
	C57BL	C57BL	C57BL	30	96
	C57BL	C57BL	Balb/c	30	83
	C57BL	Balb/c	C57BL	20	0
	C57BL	Balb/c	Balb/c	20	70

sondary' survivors gave TERTIARY CHIMAERAS. It was argued that if a change occurred in the donor tissue during its sojourn in the foreign host and, as a result, it became adapted to the host's antigen, such a change would be revealed by an increase in the number of secondary hoste which survived the wasting syndrome. The effect of serial transfer of chimaeric bone marrow on the survival of the new hosts is shown in Table III. It can be seen that the transfer of Balb/c marrow into C57BL hosts has given a consistent increase in the percentage of survivors at 50 days; it increased from 23 percent in the primary to 57 in the secondary and 96 percent in the tertiary *chimaeras*. On the other hand, when the Balb/c marrow after 50 days in the C57BL host, was returned to Balb/c mice, it failed to give the normal survival values characteristic of ISOGENIC *chimaeras* (Balb/c marrow into Balb/c irradiated host).

These findings indicate that alteration in the immunological competence of lymphoid tissue can be induced. But in order to throw more light, on the nature of changes in the donor tissue which might occur in a foreign and hostile environment, further studies are requested, in which immunological, cytological and serological methods should be combined. However, first of all, it is necessary to study the effect of irradiation on the recovery of immune response in the absence of the *thymus* gland, which has been identified as the primary organ conferring on the appropriate cells the ability to recognise foreign antigens(MILLER 1961).

Role of thymus in radiation chimaeras

Adult mice of CBA strain were thymectomized and 7 days later given a lethal dose of irradiation (LD_{99} = 850r) and injected intravenously with 5 x 10^6 haematopoietic cells from CBA donor mice. The *chimaeras* were grafted 28 days after irradiation and bone marrow therapy with two skins, both fo-

TABLE IV

The reaction of thymectomized and irradiated CBA mice to foreign skingrafts of AK and C57BL mice

	No. of mice	No. of mice showing foreign skingraft survival for		
		<20 days	<70 days	>70 days
Control group Thymectomized Non-irradiated Bone marrow injected	23	23	—	—
Experimental group Thymectomized LD_{99} irradiated Bone marrow injected	19	1	1	17

reign to the CBA host. Normal, non-irradiated CBA mice rejected such grafts within 12—14 days, due to the immunological response of the animal against the foreign antigens. It was found that the non-thymectomized *chimaeras* rejected the skin grafts of the foreign strains (Ak and C57BL) in less than 20 days, while the thymectomized *chimaeras* tolerated the grafts longer than 70 days, some even keeping them permanently. The data obtained in these experiments are given in Table IV.

In mice the results strongly suggest that for the restoration of immunological functions which have been lost due to the destruction by irradiation of cells competent to react against foreign antigens, the *thymus* is an essential organ. It appears that in the inoculum of the donor marrow the immunological potential of the primitive stem cells can be realized only when the *thymus* is present. This is the organ which confers on the stem cells the ability to recognise foreign antigens and to react against them.

These findings open up a new approach to the control of the immunological reactivity of the organism. The *thymus* may be removed or grafted in order to mitigate or eliminate the serious consequences of bone marrow therapy. Investigation in this direction is in progress.

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