EUR 702.e
REPRINT

ASSOCIATION
European Atomic Energy Community - EURATOM
Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek - TNO

TRANSPLANTATION OF VARIOUS NUMBERS OF FOREIGN BONE-MARROW CELLS IN MICE AFTER LETHAL AND SUPRALETHAL X-IRRADIATION

by

O. VOS

1965

Work performed at
the Medical Biological Laboratory RVO-TNO, Rijswijk, Netherlands

Association No. 029-63-1 BIAN

Reprinted from
INTERNATIONAL JOURNAL OF RADIATION BIOLOGY
Vol. 8, No. 1 - 1964

EUR 702.e

REPRINT

TRANSPLANTATION OF VARIOUS NUMBERS OF FOREIGN BONE-MARROW CELLS IN MICE AFTER LETHAL AND SUPRALETHAL X-IRRADIATION by O. VOS

Association : European Atomic Energy Community - EURATOM
Organisatie voor Toegepast Natuurwetenschappelijk
Onderzoek - TNO
Work performed at the Medical Biological Laboratory RVO-TNO,
Rijswijk(Netherlands)
Association No. 029-63-1 BIAN
Reprinted from "International Journal of Radiation Biology",
Vol. 8, No. 1 - 1964, pp. 21-33

Survival of irradiated mice treated with sub-optimal numbers of allogeneic bone-marrow cells could not be improved by addition of a large number of bone-marrow cells derived from the same donor strain and made incapable of proliferation by irradiation with 3000 r. This argues against the absorption of "natural antibodies" by excess of antigen.

EUR 702.e

REPRINT

TRANSPLANTATION OF VARIOUS NUMBERS OF FOREIGN BONE-MARROW CELLS IN MICE AFTER LETHAL AND SUPRALETHAL X-IRRADIATION by O. VOS

Association : European Atomic Energy Community - EURATOM
Organisatie voor Toegepast Natuurwetenschappelijk
Onderzoek - TNO
Work performed at the Medical Biological Laboratory RVO-TNO,
Rijswijk(Netherlands)
Association No. 029-63-1 BIAN
Reprinted from "International Journal of Radiation Biology",
Vol. 8, No. 1 - 1964, pp. 21-33

Survival of irradiated mice treated with sub-optimal numbers of allogeneic bone-marrow cells could not be improved by addition of a large number of bone-marrow cells derived from the same donor strain and made incapable of proliferation by irradiation with 3000 r. This argues against the absorption of "natural antibodies" by excess of antigen.

Survival-curves of mice treated with sub-optimal numbers of allogeneic bone-marrow cells showed the greatest mortality in the period of 10-20 days after lethal irradiation. This mortality was diminished if the hosts were irradiated with supralethal x-ray doses. In the combinations studied the number of required allogeneic or rat marrow cells was decreased after supralethal irradiation of the recipients.

Determinations of the number of nucleated cells present in bone-marrow and spleen showed a continuous increase in lethally irradiated mice treated with syngeneic cells and in supralethally irradiated mice treated with allogeneic cells. In lethally irradiated mice treated with allogeneic bone-marrow cells this increase was interrupted at about 14 days after irradiation by a small transitory remission.

These data were explained on the basis of an active immunological response after an extended induction period in lethally irradiated mice treated with foreign cells. After supralethal irradiation this residual response is depressed.

Stimulation of the phagocytic activity of the RES through zymosan injections did not decrease survival of lethally and supralethally irradiated mice that were treated with allogeneic or rat bone-marrow suspensions. Instead there was tendency toward a beneficial effect on survival.

Survival-curves of mice treated with sub-optimal numbers of allogeneic bone-marrow cells showed the greatest mortality in the period of 10-20 days after lethal irradiation. This mortality was diminished if the hosts were irradiated with supralethal x-ray doses. In the combinations studied the number of required allogeneic or rat marrow cells was decreased after supralethal irradiation of the recipients.

Determinations of the number of nucleated cells present in bone-marrow and spleen showed a continuous increase in lethally irradiated mice treated with syngeneic cells and in supralethally irradiated mice treated with allogeneic cells. In lethally irradiated mice treated with allogeneic bone-marrow cells this increase was interrupted at about 14 days after irradiation by a small transitory remission.

These data were explained on the basis of an active immunological response after an extended induction period in lethally irradiated mice treated with foreign cells. After supralethal irradiation this residual response is depressed.

Stimulation of the phagocytic activity of the RES through zymosan injections did not decrease survival of lethally and supralethally irradiated mice that were treated with allogeneic or rat bone-marrow suspensions. Instead there was tendency toward a beneficial effect on survival.

Reprinted from
INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, Vol. 8, No. 1, p. 21, 1964

Transplantation of various numbers of foreign bone-marrow cells in mice after lethal and supralethal X-irradiation†

O. Vos

Medical Biological Laboratory RVO-TNO, 139 Lange Kleiweg, Rijswijk, The Netherlands

(*Received* 2 *March* 1964)

Survival of irradiated mice treated with sub-optimal numbers of allogeneic bone-marrow cells could not be improved by addition of a large number of bone-marrow cells derived from the same donor strain and made incapable of proliferation by irradiation with 3000 r. This argues against the absorption of ' natural antibodies ' by excess of antigen.

Survival-curves of mice treated with sub-optimal numbers of allogeneic bone-marrow cells showed the greatest mortality in the period of 10–20 days after lethal irradiation. This mortality was diminished if the hosts were irradiated with supralethal x-ray doses. In the combinations studied the number of required allogeneic or rat marrow cells was decreased after supralethal irradiation of the recipients.

Determinations of the number of nucleated cells present in bone-marrow and spleen showed a continuous increase in lethally-irradiated mice treated with syngeneic cells and in supralethally-irradiated mice treated with allogeneic cells. In lethally-irradiated mice treated with allogeneic bone-marrow cells, this increase was interrupted at about 14 days after irradiation by a small transitory remission.

These data were explained on the basis of an active immunological response after an extended induction period in lethally-irradiated mice treated with foreign cells. After supralethal irradiation this residual response is depressed.

Stimulation of the phagocytic activity of the RES through zymosan injections did not decrease survival of lethally- and supralethally-irradiated mice that were treated with allogeneic or rat bone-marrow suspensions. Instead there was a tendency toward a beneficial effect on survival.

1. Introduction

Soon after the discovery that the therapeutic effect of inoculated haematopoietic tissue in lethally-irradiated mice is based on transplantation of proliferating cells, the number of cells required for an effective treatment was studied. It was ascertained that more allogeneic and rat cells than isogeneic cells were needed for a successful treatment (Van Bekkum and Vos 1957). In isogeneic bone-marrow suspensions, the number of stem-cells capable of proliferation determine repopulation and therapeutic results (McCulloch and Till 1960, Till and

† This investigation was supported by Euratom (European Atomic Energy Community), 51–53 Rue Belliard, Brussels, Belgium.

McCulloch 1961), but in allogeneic combinations other factors are involved (McCulloch and Till 1963). The difference in number of required syngeneic and foreign cells is generally thought to be due to an immunological reaction of the host versus the graft (Van Bekkum and Vos 1957), but no precise description of the character of this reaction has been given.

Vos, Crouch and Van Bekkum (1961) observed that a delayed foreign bone-marrow injection gave, in some respects, ameliorated transplantation results. This effect was ascribed to a lesser degree of the host-versus-graft reaction at that time. The influence of a delayed transplantation on the required number of allogeneic and rat bone-marrow cells, however, was very small and observed in the C57BL → CBA combination only.

In the present paper some factors which might influence the number of foreign bone-marrow cells required in a given combination were studied. Firstly the nature and degree of an immunological graft-versus-host reaction after lethal and supralethal radiation doses were investigated. A differentiation was made between:

(*a*) the possible occurrence of cytotoxic reactions due to natural antibodies against allogeneic transplantation antigens, and

(*b*) the presence of a residual induced immunological response after lethal x-ray doses.

In the second place the influence of the RES was considered. Wooles and Di Luzio (1962) activated the RES by zymosan injections and described a higher death-rate and shorter survival-time of these mice after lethal x-irradiation and foreign bone-marrow transplantation. For this reason the influence of the activity of the RES on the number of required allogeneic and rat bone-marrow cells for treatment after lethal and supralethal irradiation was tested in our experiments.

2. Methods

Male mice of the inbred strains (C57BL/Rij and CBA/Rij and their F_1 hybrids 8–13 weeks old and weighing 20–30 g were used as recipients. Donor bone-marrow suspensions were prepared from young adult CBA/Rij, (RF × C57BL/Rij)F_1 mice and from young rats. A few hours before bone-marrow transplantation recipients were exposed to a lethal dose of whole-body x-irradiation. Radiation constants were: 200 kv (constant potential); 18 mA; filtration 1·5 mm Cu; h.v.l. 1·8 mm Cu; distance to target 50 cm; dose-rate 46 r/min. During exposure, 10–16 mice were placed in a circular perspex container. Maximal backscatter was achieved by placing the container on a layer of 11 cm hard-board. Irradiation of bone-marrow suspensions *in vitro* was performed in the manner described previously by Smith and Vos (1962). Preparation of bone-marrow and spleen suspensions was described by Vos and Weyzen (1962). Suspensions were injected into the tail-vein without anaesthesia within a few hours after irradiation. In one experiment mentioned in the text this period was 24 hours.

Numbers of nucleated cells present in bone-marrow and spleen were estimated by preparing cell-suspensions from two femurs or from the spleen and by counting the number of cells in a haemocytometer.

Zymosan (obtained from Standard Brands Inc., New York, N.Y., U.S.A.) suspensions were homogenized in distilled water in a Potter Elvehjem apparatus and heated in a boiling-water bath for 30 min. Hyperfunction of the RES was obtained by intravenous and/or intraperitoneal injections as specified in each experiment (see § 3.3). The phagocytic activity of the RES was evaluated by determining the removal rate of intravascular colloidal carbon at different times after zymosan injections. An amount of 8 mg carbon (preparation C 11/1431a Gunther Wagner, Hanover, Germany) was injected per mouse.

3. Results and discussion

3.1. *Natural antibodies*

Cytotoxic substances against foreign cells are common in many xenogeneic combinations and, although the occurrence of such 'natural antibodies' has not been established in allogeneic combinations, very slight reactions are difficult to rule out. If minimal numbers of haematopoietic cells are injected into

Number of injected bone-marrow cells (millions)	Addition of irradiated bone-marrow cells†	
	+	−
0	15– 0	10– 0
0·2	20–10– 2	20–11– 3
0·4	10– 5– 3	10– 8– 4
0·8	20–17–14	20–20–14
3·2	10–10–10	10–10–10

† Bone-marrow cells were irradiated with 3000 r. The figures express number of surviving mice at days 0, 15 and 30 after irradiation.

Table 1. Influence of addition of lethally-irradiated CBA bone-marrow cells on number of non-irradiated CBA cells required for allogeneic bone-marrow treatment. Recipients were C57BL mice irradiated with 915 r.

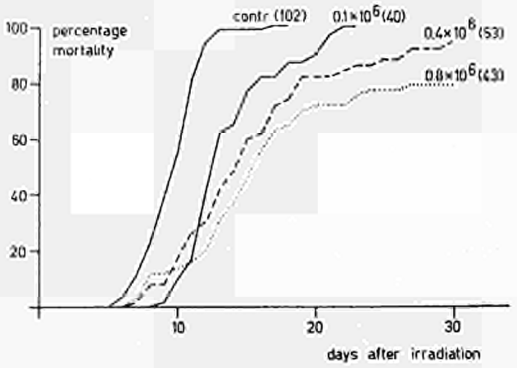


Figure 1. Survival of C57BL/Rij mice treated with various doses of CBA/Rij bone-marrow cells. X-ray dose 800 r. Figures between brackets indicate number of mice per group.

allogeneic hosts, a small number of cells is exposed to the whole pool of these antibodies, and this might influence the take of the grafted cells. This possibility was tested by addition of a large number of bone-marrow cells, made incapable of proliferation by irradiation with 3000 r, to small numbers of non-irradiated cells derived from the same donors. Survival of lethally-irradiated mice treated with these cell-suspensions was compared with survival of mice treated with the same numbers of non-irradiated cells only. The results depicted in table 1 revealed no difference in survival between both groups of mice and thus do not argue in favour of the presence of naturally-occurring cytotoxic antibodies, which can be absorbed by injected allogeneic cells.

The shape of the cumulative mortality-curves of lethally-irradiated mice treated with small numbers of allogeneic cells (figure 1) provides another argument against the occurrence of such natural antibodies. If the mortality were due to natural antibodies, this would lead to the survival of an insufficient number of allogeneic cells, resulting in either a survival-curve of the type expected for the untreated irradiated controls or in survival beyond the 30th day when secondary disease first appears. Similar results are found when small numbers of syngeneic cells are transplanted. In the present investigation, however, the survival-time of many mice has been prolonged beyond that of controls, and little or no survival is observed at 30 days after irradiation. This suggests that the cells are not killed shortly after injection, but proliferate to some extent. The production of new peripheral cells after 10–14 days, however, is insufficient to keep the mice alive much longer.

3.2. *Active immunological response*

A single whole-body irradiation provokes a considerable decrease in the capacity for a primary immunological response. Results with mice exposed to an LD_{25-30} have been described by Gengozian and Makinodan (1958). However, the immunological response of mice irradiated with a median lethal x-ray dose is still sufficient to reject allogeneic or rat bone-marrow transplants in some host–donor combinations (Van Bekkum and Vos 1957, Congdon, Makinodan and Gengozian 1957, Uphoff 1963).

After lethal radiation doses, allogeneic bone-marrow cells are generally accepted, able to proliferate and capable of producing a functional haematopoietic system, but 'reversals'—mice in which host cells take over the function of donor cells—occur (Welling, Vos, Weyzen and Van Bekkum 1959). This might indicate that a delayed immunological reaction of the recipient's immunological system is still present after whole-body irradiation with an LD_{100}. No reversals are found if the irradiation is markedly higher than the minimal LD_{100} (to be called 'supralethal irradiation'). The existence of a residual and late immunological response after lethal x-ray doses is hard to demonstrate with the usual immunological techniques, because animals die within 10–14 days after irradiation. The survival-curves of irradiated mice treated with minimal numbers of bone-marrow cells and the proliferation data of injected bone-marrow cells in our experiments provide arguments for a small immunological reaction after a lethal whole-body irradiation. This reaction is probably an important factor in determining the minimal number of foreign bone-marrow cells required.

3.2.1. *Survival of mice*

Survival-curves of mice subjected to a supralethal x-ray dose and treated with minimal numbers of bone-marrow cells are displayed in figure 2. Survival at 30 days after irradiation appears to increase with increasing number of injected bone-marrow cells. The most obvious differences with similar data obtained after a lethal (LD_{100}) irradiation (figure 1) are that (1) 30-day survival of supralethally-irradiated mice is higher in every group of mice treated with the same number of bone-marrow cells and (2) the most striking difference in death pattern of mice treated with the same dose of bone-marrow cells, but subjected to different x-ray doses, is found in the period of 10–20 days after irradiation. This last observation is further illustrated in table 2. These data suggest an

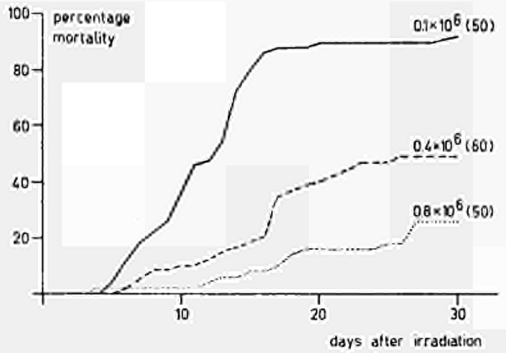


Figure 2. Survival of C57BL/Rij mice treated with various doses of CBA/Rij bone-marrow cells. X-ray dose 915 r. Figures between brackets indicate number of mice.

X-ray dose (r)	Number of mice	Percentage deaths in periods		
		0–10 days	10–20 days	20–30 days
800	176	14	71	7
915	230	15	40	13

Table 2. Death pattern of C57BL mice treated with 0·1–0·8 million CBA bone-marrow cells. Results from experiments represented in figures 1, 2 and 3 (*a*).

insufficient proliferation of small numbers of grafted bone-marrow cells to sustain life in the period between 10–20 days after a lethal irradiation. Comparison of survival at 30 days after 770 or 800 r and 915 r in some allogeneic and xenogeneic combinations is given in figure 3. In every combination smaller numbers of bone-marrow cells are required at the highest radiation dose. The effect is also present if transplantation was delayed for 24 hours after irradiation (figure 3 (*a*)). Data not represented in the graphs indicated that a 24-hour delay caused no further significant increase of survival of supralethally-irradiated mice. Some experiments performed with mice irradiated with 1030 r demonstrated that an even more effective treatment of the bone-marrow syndrome

with small doses of allogeneic bone-marrow cells could be obtained than after irradiation with 915 r. These results, however, were obscured by an early death pattern, probably due to the intestinal syndrome.

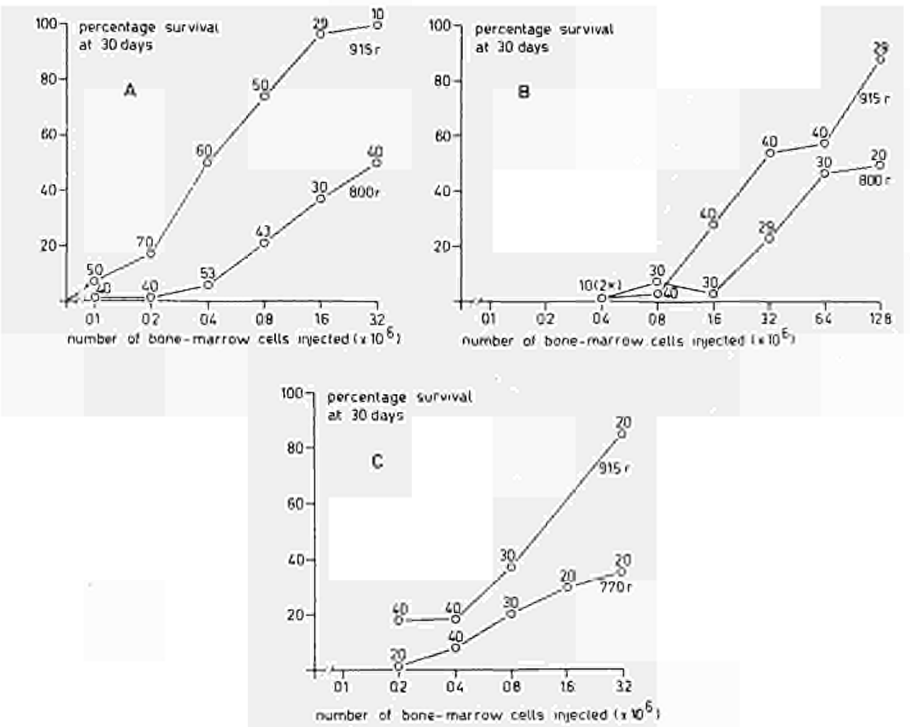


Figure 3. Survival of lethally- and supralethally-irradiated mice after allogeneic and rat bone-marrow treatment.

A: CBA/Rij → C57BL/Rij;
B: rat → (CBA/Rij × C57BL/Rij)F_1;
C: C57BL/Rij → CBA/Rij.

In experiments of A and B bone-marrow was injected within a few hours after irradiation, in experiments of C, 24 hours after irradiation. Figures indicate number of mice per point.

3.2.2. *Proliferation of haematopoietic cells*

Since the survival data of recipient mice suggested an insufficient proliferation of small numbers of grafted foreign bone-marrow cells between 10 and 20 days after irradiation with an LD_{100}, repopulation of spleen and bone-marrow was studied at various times after transplantation. After transplantation of a small number of syngeneic cells in mice irradiated with a lethal or a supralethal dose, a continuous increase of the number of nucleated cells was found (figure 4) in the spleen starting between 4 and 7 days and in the bone-marrow between 7 and 11 days after transplantation. In the spleen an overshoot was observed around the 14th day. In the allogeneic combination C57BL/Rij → CBA/Rij, the repopulation of spleen and bone-marrow was investigated after transplantation

of 0·1, 0·4 or 10 million bone-marrow cells in mice irradiated with 770 r (LD_{100}) or with 1030 r (figure 5). The results exhibit a principal difference in repopulation of mice irradiated with different doses. This difference was most evident 14 days after irradiation. Comparison of mice treated with the same number of cells but irradiated with different x-ray doses revealed that 14 days after treatment a greater number of nucleated cells was found in mice irradiated with 1030 r than in those irradiated with 770 r. This difference was significant for mice inoculated with $0{\cdot}4 \times 10^6$ and 10×10^6 cells, but not significant for the groups treated with $0{\cdot}1 \times 10^6$ bone-marrow cells. Repopulation of bone marrow and spleen started simultaneously after both radiation doses. At 10

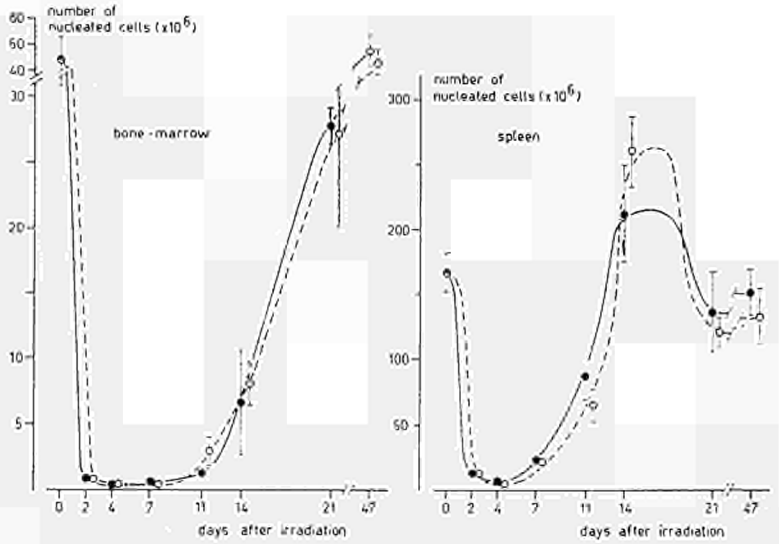


Figure 4. Repopulation of bone-marrow and spleen in lethally- and supralethally-irradiated (CBA/Rij × C57BL/Rij)F_1 mice treated with $0{\cdot}1 \times 10^6$ isogeneic bone-marrow cells. ◓ non-irradiated controls, ●—— ● lethally-irradiated mice (800 r), ○—— ○ supralethally-irradiated mice (1030 r). To facilitate drawing, this curve has been shifted a little to the right. The points indicate the mean value of groups of six mice, with standard variations.

days and later this repopulation continued in mice irradiated with 1030 r, whereas a decrease in number of nucleated cells was found in mice irradiated with 770 r. This drop was not significant in the single curves, but a significance could be demonstrated if the data of all curves were combined. Statistical methods applied were derived from Wilcoxon (1945) and Fisher (1948). These data confirm and extend earlier conclusions (De Vries and Vos 1959) inferred from pathological observations. In the last paper it was considered likely that mortality in the second half of the first month after treatment with allogeneic or xenogeneic bone-marrow was due either to the failure of the graft to take or to its secondary rejection. Our present data are in favour of the latter possibility.

3.3. *Phagocytic activity of the RES*

The influence of an activation of the phagocytic capacity of the reticulo-endothelial system by zymosan injections on transplantability of minimal numbers of foreign bone-marrow cells was studied in the combinations

$$(RF \times C57BL/Rij)F_1 \rightarrow (CBA/Rij \times C57BL/Rij)F_1$$
and rat $\rightarrow (CBA/Rij \times C57BL/Rij)F_1$.

A survey of the schemes for the treatment and the results of the stimulation

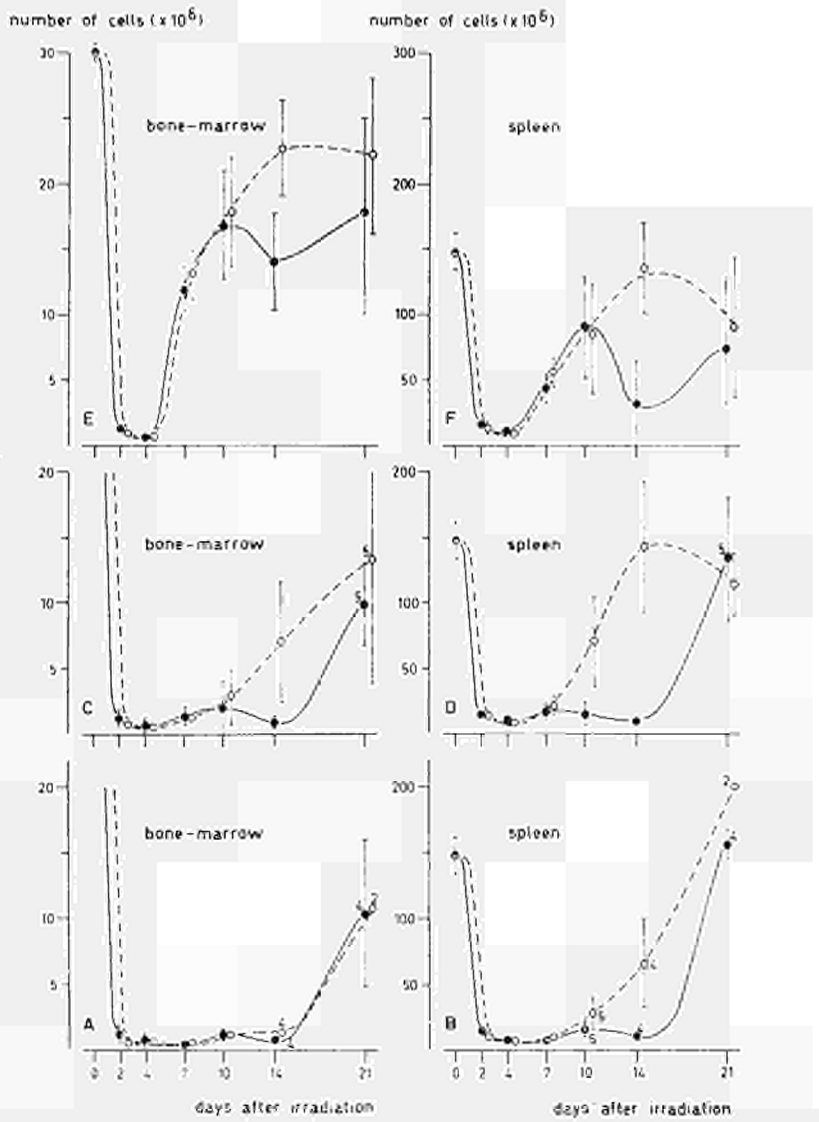


Figure 5. Repopulation of bone-marrow and spleen in lethally- and supralethally-irradiated CBA/Rij mice treated with $0{\cdot}1 \times 10^6$ (A and B), $0{\cdot}4 \times 10^6$ (C and D) and 10×10^6 (E and F) C57BL/Rij bone-marrow cells. Symbols as given in figure 4. Where figures are given they relate to mice numbers, other than six, used to establish the related point.

Experiment	Treatment†	Days after last injection on which determinations were performed	Mean values‡		
			Total number of mice	Weight spleen	Carbon removal rate $T^{1/2}$(min)
1 A	0·6 mg i.p. days 9, 8 and 7	2, 5 and 8	6	116	16
B	0·6 mg i.p. day 8	3, 6 and 9	6	101	22
2	0·6 mg i.p. days 4, 3 and 2	2, 6 and 9	9	122	15
3	0·5 mg i.p. days 4, 3 and 2	2, 5 and 9	6	160	9
4	0·5 mg i.p. days 4, 3 and 2 0·7 mg i.p. days 4, 3, 2 and 1 0·7 mg i.v. days 4, 3, 2 and 1	1, 4, 6, 8, 12 and 14	12	164	8
5	0·7 mg i.p. days 11, 10, 9, 8, 7, 3, 2 and 1 0·7 mg i.v. days 10, 8, 4 and 2	1, 4, 7 and 11	15	155	10
6	5 mg i.p. days 4, 3, 2 and 1	1, 4, 7 and 12	10	187	14
Controls	—	—	20	92	35

† Milligrams zymosan on days before irradiation, exps. 1–5 lot No. OB298, exp. 6 lot No. 5B171.

‡ Spleen-weight and carbon-removal rate in all experiments differ significantly from controls, except spleen-weight of experiment 1B.

Table 3. Zymosan treatment in different experiments and effects on RES.

is given in table 3. Stimulation of the RES is indicated by an increase in spleen weight and an increased carbon-removal rate, expressed as the time required for the carbon concentration in the blood to drop to half its original value. The

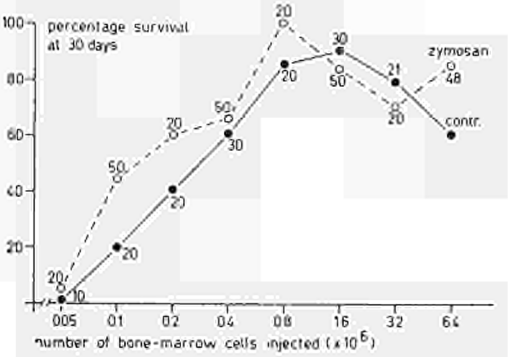


Figure 6. Effect of zymosan pretreatment on survival of (CBA/Rij × C57BL/Rij)F_1 mice treated with (RF × C57BL/Rij)F_1 bone marrow, x-ray dose 915 r. For details of zymosan pretreatment, see table 3, exps. 1 and 2 . ●—● control mice, ○—○ zymosan-pretreated mice. Figures indicate the number of mice per point.

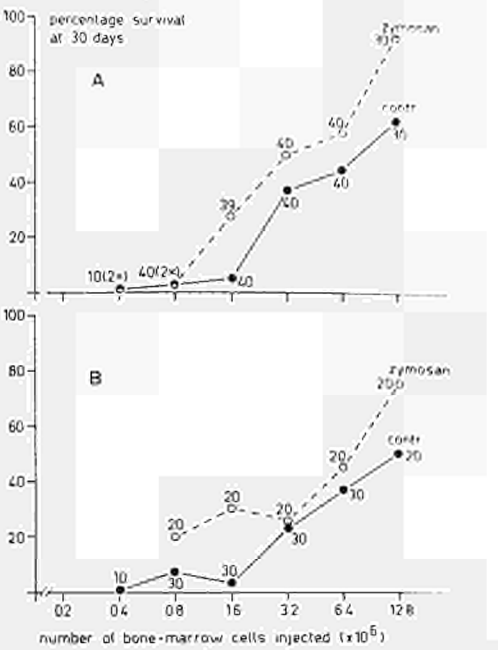


Figure 7. Effect of zymosan pretreatment on survival of (CBA/Rij × C57BL/Rij)F_1 mice treated with rat bone-marrow cells. X-ray dose 915 r (A) and 800 r (B). For details of zymosan pretreatment see table 3, exps. 3–6. Symbols as in figure 6.

phagocytic activity in our normal mice, as well as the degree of activation by zymosan treatment, was much lower than in Wooles and Di Luzio's (1962) experiments, although a considerable activation could be reached. No significant

difference in 30-day survival between zymosan-pretreated and untreated controls was observed in any of the individual experiments either after irradiation with a LD_{100} or after irradiation with a supralethal x-ray dose. The combined results of the experiments represented in figures 6 and 7 indicate a tendency towards a higher 30-day survival of the zymosan-pretreated mice, particularly in those groups which were treated with suboptimal numbers of bone-marrow cells.

4. Conclusions

Attempts to absorb cytotoxic substances in allogeneic recipients by injection of a large number of bone-marrow cells made incapable of proliferation failed to improve survival of lethally-irradiated mice treated with suboptimal numbers of cells from the same donor strain. These data together with the survival-curves of mice treated with suboptimal numbers of allogeneic cells suggested that small numbers of grafted cells were primarily accepted and that they provided an insufficient production of blood cells at a later time.

The repopulation data of bone-marrow and spleen indicate that this insufficient production of blood cells is in part due to a too-slow regeneration process after inoculation of too small a number of stem cells and partly to a decrease in repopulation around the 14th day. After injection of a large number of cells this decrease is also found, but it does not necessarily cause death of the host because of a much higher level of repopulation, which has already been established at that time. The best explanation for a decrease of the number of nucleated spleen and bone-marrow cells at about two weeks after transplantation seems to be an active immunological reaction of the host, which still occurs after lethal whole-body irradiation. The data obtained with supralethally-irradiated mice indicate that this residual immunological reaction can be further impaired by supralethal radiation doses. Final proof that this immunological reaction really exists will have to await the demonstration of the specific humoral or cellular bound antibodies.

From the results it is evident that some decrease in the number of allogeneic cells required can be obtained by increasing the radiation dose above the LD_{100} level. However, it was impossible to decrease this number of cells down to the level required for syngeneic cells. Difficulties due to the occurrence of an intestinal syndrome prevented an assessment of whether this could have been achieved after irradiation with still higher x-ray doses.

We could not confirm the results of Wooles and Di Luzio (1962), who showed that a stimulated phagocytic activity of the reticulo-endothelial system counteracts foreign bone-marrow transplantation. The degree of activation reached in our experiments was much lower than in the experiments described by the former authors, but our use of suboptimal numbers of injected cells provided us with a much more sensitive test for rejection. The differences in survival between zymosan-pretreated mice and their controls indicate, if anything, that pretreatment with zymosan has a slight beneficial effect on survival.

Acknowledgments

Our thanks are due to Dr. M. Wijnans for statistical advice and help, to Dr. H. Balner for suggestions on stimulation and determinations of phagocytic activity and to Miss L. Vermeulen, Mrs. C. Arndt and Miss J. C. Sterk for their skilled technical assistance.

Il était impossible d'augmenter la survie de souris irradiées et traitées de quantités sous-optimales de moelle osseuse allogénique par l'addition d'un grand nombre de cellules de moelle osseuse de la même souche de souris et rendues incapable à proliférer après 3000 r d'irradiation. L'absorption des anticorps naturels hypothétiques à la suite d'un excès d'antigènes est ainsi rendue improbable. Des courbes de survie des souris traitées de quantités sous-optimales de moelle osseuse allogénique montraient que la mortalité était la plus élevée pendant la période entre 10 à 20 jours après l'irradiation léthale. Cette mortalité était réduite si l'hôte fut irradié de doses de rayons-x supraléthales. Pour les combinaisons des souches étudiées ici, il fut trouvé que la quantité de moelle osseuse allogénique ou xénogénique (des rats) nécessaire pour survie, était moins si l'irradiation des receveurs était supraléthale.

La détermination du nombre de cellules nuclées se trouvant dans la moelle osseuse et dans la rate des receveurs montrait une montée graduelle pour les souris léthalement irradiées et traitées de cellules syngéniques et également en cas des souris irradiées avec une dose supraléthale et traitées de cellules allogéniques. Chez les souris léthalement irradiées et traitées de cellules allogéniques cette augmentation du nombre des cellules de la moelle montrait une rémission environ quinze jours après l'irradiation.

Ces résultats furent interprétés par la supposition d'une réponse immunologique par cettes souris léthalement irradiées et traitées de cellules allogéniques et xenogéniques soit après une longue période d'induction. Après une irradiation supraléthale une telle résponse immunologique était évidemment absente.

Une stimulation de l'activité phagocytaire du R.E.S. par des injections de zymosan ne diminuait pas la survie des souris irradiées et traitées de la moelle osseuse. Au contraire, une telle stimulation avait peut-être même un effet bénéficiaire.

Das Überleben bestrahlter Mäuse nach Behandlung mit suboptimalen Mengen homologer Konchenmarkzellen konnte nicht verbessert werden durch Hinzufügung einer großen Zahl von Knochenmarkzellen des gleichen Donorstammes die durch Bestrahlung mit 3000 r zum proliferieren unfähig gemacht waren. Diese Befunde sind unvereinbar mit einer möglichen Absorption hypothetischer natürlicher Antikörper infolge eines Übermaßes des betreffenden Antigens.

Die Überlebenskurven von Mäusen, welche mit suboptimalen Mengen homologer Knochenmarkzellen behandelt wurden, zeigten die höchste Sterblichkeit in einer Periode von 10 bis 20 Tagen nach der Bestrahlung. Diese Mortalität war geringer, wenn der Empfänger mit einer supralethalen Dosis von X-Strahlen bestrahlt wurde. In den untersuchten Stammkombinationen hat sich herausgestellt, daß die Anzahl der benötigten homologen oder heterologen (Ratte) Knochenmarkzellen nach supralethaler Bestrahlung der Empfänger abnimmt. Bestimmungen der Anzahl von kernhaltigen Zellen im Knochenmark und in der Milz der Empfänger zeigten eine allmähliche Zunahme im Falle lethalbestrahlter isolog behandelter Mäuse und auch in supralethalbestrahlten Mäusen welche mit homologen Zellen behandelt waren. Jedoch bei den lethalbestrahlten Mäusen behandelt mit homologen Knochenmarkzellen, wurde diese Zunahme der Zellularität etwa 14 Tage nach der Bestrahlung durch einen geringen vorübergehenden Rückgang unterbrochen. Diese Ergebnisse können durch Annahme einer immunologischen Reaktion erklärt werden, die nach einer längeren Induktionsperiode bei homolog oder heterolog behandelten Mäusen trotz lethaler Bestrahlung auftreten kann. Nach supralethaler Bestrahlung hingegen ist diese Restreaktion scheinbar unterdrückt.

Stimulierung der phagozytären Aktivität der R.E.S. durch Zymosaninjektionen konnte das Überleben der lethal- oder supralethalbestrahlten mit fremden Knochenmark behandelten Mäuse nicht vermindern. Stattdessen wurde eher eine Tendenz zu einem günstigen Einfluß auf das Überleben gefunden.

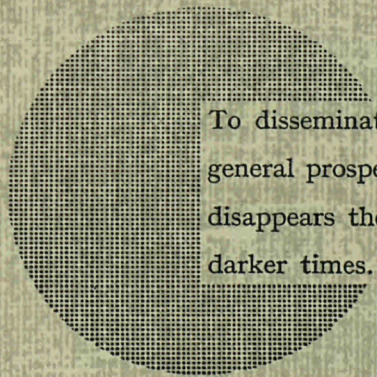
References

Bekkum, D. W. van, and Vos, O., 1957, *J. cell. comp. Physiol.*, **50** (suppl. 1), 139.

Congdon, C. C., Makinodan, T., and Gengozian, N., 1957, *J. nat. Cancer Inst.*, **18**, 603.

Fisher, R. A., 1948, *Statistical Methods for Research Workers*, 10th ed. (London : Oliver & Boyd), p. 99.

GENGOZIAN, N., and MAKINODAN, T., 1958, *J. Immunol.*, **80,** 189.
MCCULLOCH, E. A., and TILL, J. E., 1960, *Radiat. Res.*, **13,** 115 ; 1963, *J. cell. comp. Physiol.*, **61,** 301.
SMITH, L. H., and VOS, O., 1962, *Int. J. Rad. Biol.*, **1,** 143.
TILL, J. E., and MCCULLOCH, E. A., 1961, *Radiat. Res.*, **14,** 213.
UPHOFF, D. E., 1963, *J. nat. Cancer Inst.*, **30,** 1115.
VOS, O., CROUCH, B. G., and BEKKUM, D. W. VAN, 1961, *Int. J. Rad. Biol.*, **3,** 337.
VOS, O., and WEYZEN, W. W. H., 1962, *Transpl. Bull.*, **30,** 117.
VRIES, M. J. DE, and VOS, O., 1959, *J. nat. Cancer Inst.*, **23,** 1403.
WELLING, W., VOS, O., WEYZEN, W. W. H., and BEKKUM, D. W. VAN, 1959, *Int. J. Rad. Biol.*, **1,** 143.
WILCOXON, F., 1945, *Biometrics*, **1,** 80.
WOOLES, W. R., and DI LUZIO, N. R., 1962, *Amer. J. Physiol.*, **203,** 404.



To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel