Inhibition of Homograft Acceptance and Homo- and Hetero-Graft Rejection in Chimeras by Reticuloendothelial System Stimulation.* (29029)

W. R. Wooles and N. R. Di Luzio
Department of Physiology, University of Tennessee Medical Units, Memphis

Accelerated rejection of male skin isografts by female recipients(1) and rejection of homologous and heterologous bone marrow transplants in lethally irradiated mice pretreated with zymosan or glucan(2) is dependent in part, upon the functional state of the reticuloendothelial system (RES) of the host existing at time of transplantation. The mechanisms underlying this rejection phenomenon are unknown, although accelerated rejection of male skin isografts is thought to be mediated by an enhanced immune response (1). Similarly, zymosan, glucan, or Bacillus Calmette-Guérin (BCG), excellent RE stimulating agents, enhance non-specific defense mechanisms of the host, increase host resistance to transplants of various homologous tumors(3) and markedly increase antibody formation(4,5).

A hyperfunctional state of the RES, induced prior to radiation exposure by zymosan, or by an active fraction of zymosan, glucan(6), has been demonstrated to be unaltered after exposure to lethal radiation(7). Since the radiation recovery effect of genetically foreign bone marrow was abolished by an enhanced functional state of the RES(2), and since the major site of distribution of foreign bone marrow following transplantation is essentially within the RES(8,9), it became of interest to evaluate the relation between phagocytic activity of the RES and survival of lethally X-irradiated mice treated with homologous bone marrow.

Although the etiology of secondary disease in chimeras is not yet completely established, it is generally agreed that there is an underlying immunological mechanism. However, depending upon the transplantation system employed, this immune response is mediated either by the host against the transplanted tissue or by the transplant against the host (10). In view of the fact that pre-irradiation induced stimulation of the RES is associated with a lack of acceptance of genetically foreign bone marrow(2), the effect of RE stimulation upon the survival of lethally irradiated mice bearing apparently successful foreign marrow transplants was evaluated.

Materials and methods. Male C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine) weighing between 20-25 g were employed as bone marrow recipients and were allowed food and water *ad libitum*. All bone marrow recipients received 800 r whole body ionizing radiation according to factors previously described(2). Prior to X-irradiation, experimental mice received glucan(6) or trypsinized zymosan(6) daily in the amount of 4 mg/100 g body weight as a saline suspension until varying levels of RE activity were obtained. Control animals received equivalent volumes of saline. The phagocytic activity of the RES was evaluated in randomly selected irradiated animals of each group by measuring the intravascular removal rate of a suspension of colloidal carbon in the amount of 16 mg/100 g body weight(7). Femoral bone marrow was obtained from adult male $B6AF_1/J$ donor mice (C57BL/6J $\times$ A/J). Marrow suspensions containing 5 $\times$ 10^7 viable cells in a volume of 0.5 ml were injected into control and RE stimulated recipients within 2 hours after radiation exposure. Viability of the marrow cell suspension was determined by staining with 0.01% trypan blue. Irradiated control mice received equivalent volumes of saline.

Radiation chimeras were male C57BL/6J mice which had received 800 r X-irradiation and were transplanted with either heterologous, homologous or isologous bone marrow. Isologous bone marrow was obtained from

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TABLE I. Organ Weights and Phagocytic Function of X-irradiated Mice Prior to Transplantation of Homologous Bone Marrow.

Group	Body wt (g)	% body wt			Colloidal carbon removal t/2 (min.)
		Liver	Lung	Spleen	
I	20	5.12	.67	.32	11.55
	± 1	± .18	± .01	± .01	± .25
II	20	5.63	1.49	.67	6.13
	± 1	± .22	± .05	± .04	± 2.06
III	20	5.77	.81	.73	4.39
	± 1	± .23	± .10	± .18	± .50
IV	22	6.33	.89	.81	1.85
	± 1	± .19	± .03	± .04	± .06

Means ± standard error derived from 3-6 animals per group.

femurs of male C57BL/6J mice of the same age and weight and was injected in the amount of 4×10^6 cells per recipient. Homologous bone marrow was prepared as described above and the number of cells injected was 5×10^7 per recipient. Heterologous bone marrow was obtained from the femurs of male albino rats (Holtzman Co., Madison, Wisc.) weighing approximately 100-150 g and was injected in the amount of 2×10^8 cells per recipient. Radiation chimeras, which had survived 30 days or longer, were injected intravenously with either saline or glucan in the amount of 4 mg/100 g daily for 8 days and the 30 day survival was recorded.

Results. The functional state of the RES of representative X-irradiated mice from the various groups receiving the homologous bone marrow transplant is presented in Table I. Group I represents saline-treated X-irradiated mice with a normal intravascular removal rate of colloidal carbon. In the glucan-X-irradiated Group II, no increase in liver weight was observed whereas spleen and lung weight were increased 122% and 110%, respectively. The intravascular removal rate of colloidal carbon was enhanced 2-fold in this group. The degree of hyperphagocytosis of animals which had received trypsinized zymosan (Group III) is indicated by the mean t/2 of 4.4 min, or an approximate 3-fold increase in the colloidal carbon removal rate over the control group. No increase in liver and lung weight was observed whereas spleen weight was increased 130%. Those animals in Group IV, subjected to more prolonged glucan administration, demonstrated a marked increase in phagocytic activity as indicated by a 6-fold enhancement in phagocytic function. Pronounced RE organ hypertrophy is evidenced by the 24%, 33% and 153% increase in weights of liver, lung, and spleen, respectively.

As the functional state of the RES of the animal is increased, the 30 day survival of lethally irradiated mice transplanted with homologous bone marrow is decreased (Fig. 1). At normal phagocytic activity, indicated by a mean colloidal carbon t/2 of 12 min, survival is approximately 85%. As RE activity increases, survival of mice treated with homologous bone marrow progressively decreases until at maximal RE activity, indicated by mean t/2 of 1.8 min, 100% mortality is manifested.

Radiation chimeras, bearing successfully transplanted isologous bone marrow, were unaffected by RE stimulation (Table II). Administration of glucan to heterologous chimeras was associated with apparent rejection of the transplant as indicated by the subsequent death of all recipients as contrasted to a 30 day survival of 58% in the saline-

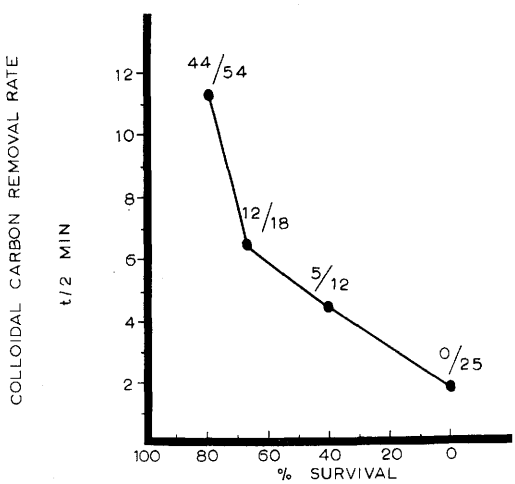

FIG. 1. Relation between phagocytic activity of the RES and 30 day survival of X-irradiated C57BL/6J mice transplanted with homologous (B_6AF_1/J) bone marrow. Figures above each point represent number of animals surviving 30 days to number originally in each group.

TABLE II. Effect of RE Stimulation on Survival of Radiation Chimeras.*

Treatment	Bone marrow transplanted	No. survived/ No. group	% 30 day survival
Saline	Isologous	5/5	100
Glucan	"	4/5	80
Saline	Homologous	12/12	100
Glucan	"	11/21	52
Saline	Heterologous	7/12	58
Glucan	"	0/20	0

* All mice were C57BL/6J radiation chimeras which had survived 30 days or longer.

treated heterologous chimera group. Glucan administration was associated with a 52% survival in the homologous chimera group whereas no mortality was observed in saline-treated homologous chimeras.

Discussion. Intravenous administration of glucan or trypsinized zymosan is associated with marked hyperplasia of those organs containing predominantly RE cellular elements (2, 5-7, 11, 12). This proliferative response is associated with increased hepatic DNA synthesis limited to endothelial cells(13) and an increase in size and number of Kupffer and splenic reticulum cells(10,12). That the induced hyperactivity is associated specifically with the stimulation of hepatic littoral cells has been demonstrated by the findings of an unaltered bromsulphalein retention in RE hyperfunctional rats(12).

The tissue distribution of homologous bone marrow, in the early time periods after transplantation to irradiated hosts, has been demonstrated to be essentially within the RES (8,9). We have previously demonstrated that a hyperfunctional state of the RES, induced by glucan or trypsinized zymosan, existing prior to X-irradiation, is unaltered up to 4 days after exposure, at which time saline control animals manifested marked RE depression(7). In view of these observations it is possible that the lack of acceptance of homologous bone marrow in irradiated RE hyperfunctional mice, as indicated by the reduced survival, may be due to enhanced phagocytosis and subsequent intracellular digestion of the transplanted marrow cells.

Cutler has demonstrated that activation of the RES with zymosan is associated with a marked increase in antibody formation(4). Similar findings of enhanced hemolysin and bacterial agglutination titers in mice infected with BCG has been reported by Halpern (14). The accelerated rejection of male skin isografts by BCG treated female recipients was concluded to be due to an enhanced immune response(1). It has also been previously demonstrated that RE stimulation does not alter the high degree of radiation recovery afforded by isologous bone marrow, whereas no recovery was observed in similar groups of RE hyperfunctional mice receiving either homo- or heterografts(2). These findings suggest possible immunological involvement in the lack of acceptance of the homograft by RE hyperfunctional X-irradiated mice. In view of the fact that phagocytic activity and immunological responsiveness are intimately correlated(4,5,14) it is possible that graft rejection in RE- hyperfunctional irradiated mice may be due to a combination of these factors. The decreased survival observed in glucan-treated homologous and heterologous radiation chimeras indicates that RES can be involved in the rejection phenomenon. However, it is not presently known how glucan administration induces rejection. Based upon the observations of Gengozian *et al,* it appears that the secondary response observed in heterologous chimeras is due to an immunological response mediated by the host against the grafted marrow(15,16). The finding that nearly 50% of the saline-treated heterologous chimeras died during the 30-day observation period indicates that an immune reaction was occurring naturally and that RE stimulation possibly accelerates this reaction.

The fact that RE stimulation, while ineffective in altering survival of the isologous bone marrow-treated group, was capable of causing the death of 50% of the homologous chimeras as opposed to 100% mortality in heterologous chimeras, indicates that the severity of the reaction is related to the antigenicity of the transplanted tissue.

It cannot be concluded from the radiation chimera data reported in this study that the apparent rejection of successfully trans-

planted homologous and heterologous bone marrow is due either to a host *vs* graft response or to a graft *vs* host response since glucan could conceivably induce the transplanted donor cells to react against the host tissue. Indeed, Balner(17) observed that the host-type peritoneal macrophages of radiation chimeras are replaced by donor-type macrophages. Evidence is not currently available to indicate whether the fixed macrophages remain as host, or like free peritoneal macrophages, become donor in genetic origin. Until this information is available it cannot be established whether donor or host RE cells were stimulated by glucan.

Recent findings by Zeiss and Fox indicate that the host is responsible for approximately 60% of the RE hyperactive state and splenomegaly observed in the graft *vs* host reaction (18). On the basis of these observations, it is possible that the secondary disease is the result of an interaction between the host and the grafted tissue and is not mediated by either exclusively. It is apparent from the results of these studies that the RES may be involved in determining the early acceptance or rejection of homologous bone marrow as well as a factor in the secondary disease phenomenon.

Summary. The subsequent survival of lethally X-irradiated mice transplanted with homologous bone marrow is dependent, in part, upon the functional state of the RES existing at time of transplantation. As phagocytic activity of the RES is increased, incidence of mortality is similarly increased. RE stimulation of heterologous chimeras is associated with the subsequent death of the host. The lesser degree of mortality observed in homologous chimeras subjected to RE stimulation suggests that the severity of the reaction may be related to the degree of antigenicity of the transplanted marrow. This is further supported by the observation that RE stimulation does not effect the survival of isologous chimeras.

1. Balner, H., Old, L.J., Clarke, D. A., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 58.

2. Wooles, W. R., Di Luzio, N. R., *Am. J. Physiol.,* 1962, v203, 404.

3. Old, L. J., Clarke, D. A., Benacerraf, B., Goldsmith, M. M., *Ann. N. Y. Acad. Sci.,* 1960, v88, 264.

4. Cutler, J. L., *J. Immunol.,* 1960, v84, 416.

5. Wooles, W. R., Di Luzio, N. R., *Science,* 1963, v142, 1078.

6. Riggi, S. J., Di Luzio, N. R., *Am. J. Physiol.,* 1961, v200, 297.

7. Wooles, W. R., Elko, E. E., Di Luzio, N. R., *Rad. Res.,* 1962, v16, 546.

8. Brown, N. B., Hirsch, B. B., Nagareda, C. S., Hockstetter, S. H., Laraghan, W. G., Toch, P., Kaplan, H. L., *J. Nat. Cancer Inst.,* 1955, v15, 949.

9. Odell, T. T., Smith, L. H., *Acta Haematol.,* 1958, v19, 114.

10. Gengozian, N., In *Ionizing Radiation and Immune Processes,* ed. C. Leone. Science Publisher, New York, 1962, p403.

11. Ashworth, C. T., Di Luzio, N. R., Riggi, S. J., *Exp. Mol. Path.,* 1963, Supp. 1, p83.

12. Riggi, S. J., Di Luzio, N. R., *Nature,* 1962, v193, 1292.

13. Kelly, L. S., Dobson, E. L., Finney, C. R., Hirsch, J. D., *Am. J. Physiol.,* 1960, v198, 1134.

14. Halpern, B. N., *J. Pharm. Pharmacol.,* 1959, v11, 321.

15. Gengozian, N., Makinodan, T., *Cancer Res.,* 1957, v17, 970.

16. Gengozian, N., Peterson, W. J., Makinodan, T., *ibid.,* 1959, v19, 534.

17. Balner, H., *Transplantation,* 1963, v2, 217.

18. Zeiss, I. M., Fox, M., *Nature,* 1963, v197, 673.

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