PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 628–629 (1975)

Modification of Graft-versus-Host Disease with Con A and Preimmunization[1] (39093)

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Graft-versus-host disease (GVHD) is one of the major obstacles to the routine successful transfer of bone marrow cells between histoincompatible individuals. Allogeneic bone marrow contains mature T-lymphocytes that upon engraftment are able to recognize and react against the foreign transplantation antigens of the host, producing a syndrome of acute or delayed morbidity and mortality as a result of a graft-versus-host immune reaction (1, 2). This has limited the use of bone marrow grafts in man to exchanges between individuals closely related antigenically; however, even in those cases where there is apparent HL-A compatibility, some degree of GVHD is common (3). It is clear, therefore, that the scope and efficacy of bone marrow therapy would be greatly enhanced if a way could be found to eliminate from bone marrow preparations those mature lymphocytes that produce GVHD without adversely affecting the erythroid, myeloid, and lymphoid stem cells.

Previous work (4) has shown that when allogeneic murine bone marrow and spleen cell preparations are incubated with concanavalin A (Con A) at concentrations sufficient to kill or inhibit mature T cells and then are injected into irradiated hosts, the majority of stem cells survive to protect the recipients against the effects of the radiation, and GVHD either is prevented entirely or the incidence is greatly reduced. Overall, 84% of 140 mice given Con A treated cells were alive and healthy 100 days after irradiation whereas none of the mice given untreated cells survived (mean survival time, 16.2 days). While these results were encouraging, nonspecific toxic effects limited the concentration of Con A that could be used to 300 μg/ml or less and thus precluded the elimination of all T cells from heavily contaminated marrow specimens without risking severe damage to vital stem cell populations. In an attempt to increase the survival of recipients of marrow preparations heavily contaminated with mature T-lymphocytes, mice were immunized with sheep RBC (SRBC) 3 days before radiation in an effort to activate suppressor T cells (5); after irradiation they were injected with large numbers of Con A treated allogeneic marrow and spleen cells. The results of these experiments suggest that the activation of host suppressor T cells may be a valuable adjunct in the prevention of GVHD induced by foreign T-lymphocytes.

Materials and methods. The hosts were adult male B6D2F$_1$ mice. They were given 900 rads of whole body γ radiation on the day of cell transfer (^{60}Co; 120 rads/min; target-source, 80 cm; 100% lethal to unprotected mice in 11–14 days). After irradiation the mice were housed five per cage in a conventional open colony; they were given neomycin water for 21 days. Certain groups of mice were given a single injection (0.2 ml) of 20% SRBC ip 3 days before they were irradiated.

Bone marrow and spleen cell donors were adult male C57BL/6 mice. Cell suspensions were prepared by standard methods (4), and each recipient was given 5×10^6 bone marrow and 6×10^6 spleen cells iv or ip. Aliquots of these cell suspensions (55×10^6/ml) were incubated with Con A (lot 89, Miles Laboratories, Kankakee, Ill.) at a concentration of 300 μg/ml for 30 min at room temperature. The cells were not washed before injection in a volume of 0.2 ml (each mouse received 60 μg Con A).

Results and discussion. None of the mice given 3.25 or 11×10^6 untreated parental cells iv survived 100 days (MST 34.2 and 13.3 days), and only 19% of those injected ip were alive at the end of this time (Table I). The reduction in mortality associated

[1] This work was supported by USPHS Research Grants HD 07708, DE 02668, and RR 5333.

TABLE I. SURVIVAL OF LETHALLY IRRADIATED B6D2F$_1$ MICE GIVEN PARENTAL BONE MARROW AND SPLEEN CELLS

Host treatment[a]	Number of Cells ($\times 10^6$) Bone marrow	Spleen	Treatment[b]	Route cells given	One-hundred-day survival (number/total)	Mean survival time (days)[c]
					0/20	11.4
	0.75			iv	0/10	18.3
	1.25	2.0		iv	0/9	34.2
	5.0	6.0		iv	0/30	13.3
	5.0	6.0		ip	5/26	56.1
SRBC	5.0	6.0		iv	0/12	58.6
SRBC	5.0	6.0		ip	6/18	85.2
	5.0	6.0	Con A	iv	25/30	47.5
	5.0	6.0	Con A	ip	28/30	32.5
SRBC	5.0	6.0	Con A	iv	20/20	
SRBC	5.0	6.0	Con A	ip	20/20	

[a] Three days prior to radiation these mice were given 0.2 ml 20% SRBC ip.
[b] The marrow and spleen cells were incubated with Con A 300 μg/ml.
[c] Mean survival time of mice that died before the 100th postradiation day.

with ip *versus* iv injection of allogeneic cells has been noted previously (4), and although there is no clear explanation for this difference, one possibility is that T cells injected ip may first recognize and attack host peritoneal cells rather than the more vital populations of liver, marrow, and lymphoid tissues that would be encountered if they had been given iv. However, it should also be noted that ip injection is significantly less efficient than the iv route in the transfer of erythroid and myeloid stem cells (6).

Preimmunization of the hosts with SRBC alone provided only minimal protection against the GVHD induced by untreated parental cells. None of the immunized mice injected iv survived 100 days, although the MST was significantly increased (58.6 *versus* 13.3 days), and only a modest increase in the survival rate was noted when the cells were given ip (33% versus 19% for the nonimmunized controls).

When parental cells were incubated with Con A before injection, 83% (iv) and 93% (ip) of the unimmunized recipients were alive and healthy 100 days later. The same cells produced no deaths when injected into 40 preimmunized recipients.

These and previously reported results (4) clearly establish that at appropriate concentrations the immunosuppressive properties of Con A (7) can be used to prevent or greatly reduce the incidence of fatal GVHD in models designed to produce mild or moderately severe disease. In addition, the data presented here suggest that the activation of host suppressor T cells by means of immunization prior to irradiation (5) further reduces the risk of GVHD in recipients of Con A treated allogeneic cells. Thus, in these experiments a very severe form of GVHD was prevented by the additive actions of these two treatment modalities.

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Received July 3, 1975. P.S.E.B.M. 1975, Vol. 150.