Inhibitory Effect of α-globulin on the Second Set Allograft Reaction (36597)

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(Introduced by A. L. Barron)

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It has been shown that plasma fractions containing high levels of α-globulins cause a significant prolongation of primary skin allografts survival times (1–3).

The effect of α-globulin fraction isolated from normal human plasma on second set skin allograft reaction was investigated in rats and mice, and the results are presented here.

Materials and Methods. Animals. Hooded Lister male semi-inbred rats were used as donors of skin grafts to white male semi-inbred rats of a strain "Sabra" developed at the Hebrew University, Jerusalem. Male C57Bl mice were used as donors of skin grafts to male Balb/c mice. Rats and mice weighed 120–150 g and 20–25 g, respectively.

Preparation of α-globulin fraction. α-Globulin was prepared from normal human plasma following the method previously described by Mannick and Schmid (3).

Skin grafting procedure. The technique for skin grafting was the same as previously reported (4).

Transplantation and treatment experiments. "Sabra" rats received skin grafts from Hooded Lister rats and were divided into 3 groups: The first group was injected iv with 20 mg of α-globulin in 0.5 ml of saline each, 30 min before grafting. Each animal in the second group was treated as above and 20 days later received a second graft from the corresponding donor. Animals in the third group received primary grafts and 20 days later were injected with α-globulin immediately followed by second grafts. Control groups of animals were grafted and treated with saline or with 20 mg of human serum albumin (HSA; Magen David Adom, Blood Services, Tel Aviv-Jaffa) at the same time that their counterparts received α-globulin. The grafts were inspected daily and considered to be rejected when completely hard and necrotic.

Transfer experiments. Balb/c mice were actively immunized by the transplantation of single skin grafts from C57Bl mice. Speens were removed from the primary hosts after these allografts had been fully destroyed. Spleen cell suspensions were incubated with α-globulin or with HSA (4 mg/10^6 cells) at 37° for 30 min. Aliquots of these suspensions were also incubated with Veronal buffer (pH 7.4) as controls. After incubation the cell suspensions were centrifuged at 700 rpm for 5 min and the supernatant fluids were discarded. The cells were resuspended in Veronal buffer.

Secondary Balb/c hosts were each inoculated ip with a suspension containing 2×10^8 cells in 0.5 ml of Veronal buffer. These animals were transplanted with skin grafts from C57Bl donor mice 3 days after the cell transfers.

The effect of α-globulin on the viability of spleen cells was assessed by trypan blue exclusion as described elsewhere (5).

Results. The effect of α-globulin on the first and second set skin allograft reactions in rats is shown in Table I. Animals in group 1 showed prolongation of graft survival times following treatment with α-globulin. Examination of the second grafts in group 2 animals showed suppression of rejection when α-globulin was injected at the time of the first grafts. Administration of α-globulin just prior to the second grafts (group 3) resulted in rejection of these grafts similar to a first set

TABLE I. Effect of α-Globulin on the First and Second Set Skin Allograft Responses in Rats.

			Graft survival times			
			α-Globulin		Control[a]	
Group	Skin graft			MST[b]		MST
1	1st set	Days	10 11 12 20 24 26	17.7 ± 2.0	8 9 10 11 12	9.3 ± 0.28
		No. of animals	2 1 1 3 2 1		7 5 4 3 1	
2	2nd set	Days	8 9 10 11 12	9.4 ± 0.42	5 6	5.6 ± 0.11
		No. of animals	3 3 2 1 1		8 12	
3	2nd set	Days	8 9 10 11 12	9.8 ± 0.46	5 6 7	5.8 ± 0.2
		No. of animals	2 3 2 1 2		8 8 4	

[a] Data shown are for rats receiving HSA. When saline was used the MST for group 1 was 9.5 ± 0.31; group 2, 5.4 ± 0.1; and group 3, 5.7 ± 0.17.

[b] MST = mean survival time ± SE.

reaction.

In other experiments performed with mice it was found that sensitized Balb/c spleen cells incubated with α-globulin failed to transfer immunity against C57Bl skin transplants to isologous recipients (Table II). The mean survival time (MST) of the donor grafts on these recipients was 11.8 ± 0.59 days analogous to 12 ± 0.85 days, which was the MST of primary C57Bl skin grafts on Balb/c mice. However, an immune reaction was transferred in control experiments by sensitized spleen cells incubated with HSA or Veronal buffer instead of α-globulin. The MST of the donor grafts in this case was 7.1 ± 0.31 days analogous to 7.3 ± 0.27 days which was the MST of secondary grafts.

The viability of α-globulin treated cells was indistinguishable from that of cells treated with HSA or Veronal buffer as measured by the trypan blue method.

Discussion. The inhibitory effect of α-globulin fraction obtained from normal human plasma on rejection of primary skin allografts as previously reported (1–3) was confirmed in our experiments in rats. Our studies were extended to show the effect of α-globulin on second set response. A marked suppression of second set allograft rejection was observed when α-globulin was injected either before the first graft was administered or even before the second graft. In explaining the mode of action of the α-globulin fraction in the first instance, it is possible that the material could have prevented the natural sequence of immune reaction by inhibiting the first set allograft reaction. A second allograft from the same donor would therefore be dealt with as a first set allograft and would not be rejected in an accelerated pattern, as was observed in our experiments. In the second case the α-globulin was apparently able to interfere

TABLE II. Effect of α-Globulin on the Ability of Sensitized Mouse Spleen Cells to Transfer Immunity to Skin Allografts in Isologous Recipients.

	Graft survival times			
	α-Globulin-treated cells		HSA-treated cells[a]	
		MST[b]		MST
Days	9 10 12 13 14	11.8 ± 0.59	6 7 8 9	7.1 ± 0.31
No. of animals	1 3 1 3 2		3 4 2 1	

[a] MST for saline-treated cells was 7.2 ± 0.28.

[b] MST = mean survival time ± SE.

with second set rejection after a primary response, and, thereby, suggesting different mechanism of action. Transfer experiments of spleen cells from mice sensitized by skin grafts further confirmed that the secondary immune response to allografts could be suppressed by α-globulin.

The experiments in which no suppression of the immune response was detected when similar amounts of HSA was used instead of the α-globulin preparation, seem to rule out the possibility that α-globulin may act simply by antigenic competition. Moreover, α-globulin did not appear to act by cytotoxicity as the α-globulin-treated spleen cells were as viable as untreated cells determined by the trypan blue exclusion test.

The present studies confirm those of Glasgow *et al.* (6, 7) and ourselves (5, 8), using sheep red blood cells instead of skin grafts and differ from those reported by Mowbray (2) who found no inhibition of second set rejection of skin allografts in rats and rabbits using similar α-globulin fraction. The latter investigator used α-globulin from bovine serum rather than from human serum, as used in the present studies, the difference in results may be attributed to the species origin of the α-globulin fractions.

Summary. α-Globulin fraction prepared from normal human plasma suppressed first as well as second set skin allograft reactions in rats.

Spleen cells taken from mice, immunized by transplantation of skin allografts, and incubated with the α-globulin fraction failed to transfer transplantation immunity to isologous recipients.

No evidence was obtained for cytotoxicity of α-globulin. Since human serum albumin failed to suppress immune response the mode of action of α-globulin was not considered to be due to antigenic competition.

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