Studies on Induction of Tolerance of the Y Chromosome Antigen by Spleen Microsomes.* (30157)

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We have previously shown that microsomal fractions from spleen cells function as potent transplantation antigens. This was demonstrated by their capacity to induce in allogeneic recipients accelerated rejection of skin grafts from the microsome donor(1,2). In view of this observation it became of interest to test the possibility whether a defined subcellular fraction (microsomes) could function as antigen for induction of tolerance. The use of cell free antigenic preparations in this manner could eliminate graft *vs* host reaction and runting disease which complicate tolerance to allogeneic tissues induced by viable lymphoid cells(3). The finding of Eichwald and Silmser(4) that in mice an isoantigen associated with the Y chromosome function as a weak histocompatibility barrier offered a simple assay to test this possibility. In this system, tolerance of the Y chromosome antigen has been induced by inoculation of lymphoid cells from male mice into newborn or adult females(5,6). Recently it has also been reported that tolerance across the Y chromosome antigens can be induced by inoculation of cell free tissue extracts or subcellular fractions obtained from male cells into newborn or adult females(7-9).

The present study will present evidence to show that microsomal fractions prepared from spleens of syngeneic males can induce tolerance of the Y chromosome antigen in neonate female C57 mice. Moreover, repeated administration of such microsomal preparations to adult females will prolong the expected survival times of male skin grafts on some of the treated females.

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Methods. Mice of the C57BL/6 strain were used throughout this study. In this strain, female mice have been shown to reject syngeneic male skin grafts with regularity, the median survival times of these grafts being in the range of 27 days(10). The skin grafts were fitted suprapannicular 15 mm in diameter applied to the dorsal thoracic region. The grafts were covered with nonadhering dressing and latex bandage then wrapped with adhesive tape. Each female received skin from its own male littermate. The mice used in this study were over 2 months old at the time the skin grafts were applied.

Preparation of microsomal fraction. Freshly excised spleens from male C57 mice were homogenized in 0.25 M sucrose then centrifuged at 900 $\times$ g for 10 minutes. The supernatant was saved and the sediment rehomogenized in 0.25 M sucrose and centrifuged as above. The supernatant from these 2 extractions was pooled and then centrifuged in Spinco rotor #40 at 10,000 $\times$ *g* for 10 minutes to remove the mitochondrial fractions. Microsomal fractions were then sedimented from this supernatant in the same rotor at 105,000 $\times$ *g* for one hour. All steps were carried out at 4°C. Microsomal suspensions were made up in saline for injection. For i.v. injection 0.1 mg of Connaught heparin® per mouse was added.

Schedule of microsomal injection. Newborn mice, when 5 days old received either a single i.p. injection of microsomes, or 4-12 biweekly microsomal injections. The latter group received the first 10 injections by the i.p. route and the last 2 were given intravenously. One group of adult females was given a single i.v. injection of microsomes followed 3 days later by a syngeneic male skin graft. In another group of adult females given 10 such injections, the skin grafts were applied following the second or third injection.

Criteria for graft acceptance. Normal fe-

TABLE I. Survival of Syngeneic Male Skin Graft on Female C57 Mice Injected Repeatedly with Syngeneic Male Spleen Microsomes During Neonatal Life.

Group	Age at inj	No. of inj	Dose (spleen equivalent)	Viable grafts/total on day 50	90	360
1	adults*	0	0	1/19	1/19	0/19
2	5 days	12 (i.p. & i.v.)	½, ½, 10 × 1	8/12	8/12	4/12†
3	5 "	1 (i.p.)	1	0/8		
4	5 "	4 (i.p.)	1	0/4		

* Control.

† After the grafts have been in residence for 270 days each tolerant female in this group received an i.p. injection of spleen cells obtained from 3 normal female C57 mice.

Groups 2, 3 and 4 were skin grafted when 2 mo old.

male C57BL/6 mice may accept a syngeneic male skin graft for up to 3 months. Thus female C57 mice bearing male skin grafts with good coats of hair for more than 100 days were considered to be tolerant.

Results and observations. Induction of tolerance by repeated inoculation of newborn females with microsomes from syngeneic male spleens. Intravenous injection of microsomal suspension was invariably fatal during the first 24 hours of life. The intraperitoneal route also resulted in a high degree of mortality when microsomes were injected prior to the 5th day of life. For these reasons, microsomal suspensions were injected on the 5th day of life by the i.p. route.

It may be seen from Table I that tolerance of male skin grafts was induced in 4 of the 12 females which received 12 injections of male spleen microsomes shortly after birth. The grafts on these mice are still viable 360 days since their application. These tolerated grafts grew iron grey coats of hair upon acceptance of the grafts which has persisted till the present time. In the same group another 4 females have accorded their male skin grafts prolonged survival times up to 90 days, but eventually these grafts were destroyed within 100 days. It was of interest in this connection to test for ability of normal syngeneic female lymphoid cells to reject tolerated grafts. Each of the tolerant females was given an i.p. injection of spleen cell suspension from 3 normal female C57 mice 270 days after the grafts were applied. These grafts are still viable and in good condition 100 days later (Fig. 1).

In a group of 8 females a single i.p. injection of microsomes on the fifth day of life failed to produce tolerance or prolong survival times of male skin grafts. This was also observed in another group of 4 females given 4 biweekly injections of microsomes.

Survival of syngeneic skin grafts on adult female C57 mice treated with i.v. male spleen microsomes. The results were uniformly negative in adult animals (Table II). Despite repeated administration of spleen microsomes by the i.v. route, none of the adult females treated in this fashion accepted male skin grafts permanently. However, the pooled results indicate that 5 of the 20 recipients accorded the male skin grafts prolonged survival times (50 days) whereas only one of 19 control grafts survived this long.

Discussion. The results demonstrate that tolerance of the Y chromosome antigen can be induced by repeated inoculation of male

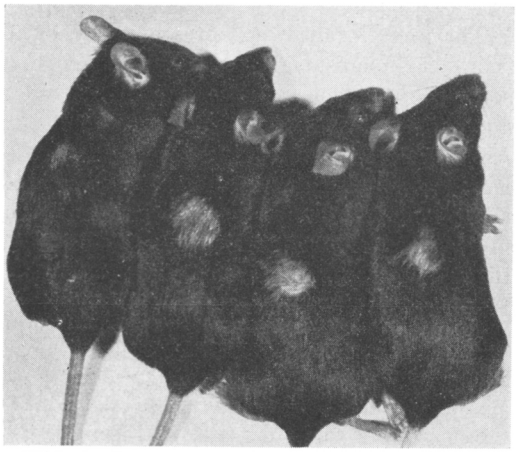

FIG. 1. Syngeneic male skin grafts on C57BL/6 females one year after grafting. Tolerance was induced by repeated injections of male spleen microsomes beginning during neonatal life.

TABLE II. Survival of Syngeneic Male Skin Grafts on Female C57 Mice Inoculated Repeatedly with Syngeneic Male Spleen Microsomes During Adult Life.

Group	Age at inj	No. of inj	Dose (spleen equivalent)	Viable grafts/total on day 50	60	90	100
1	adults*	0	0	1/19	1/19	1/19	0/19
2	adults	10 i.v.	$2, 9 \times 1$	4/11	2/11	0/11	
3	"	10 i.v.	$3, 3, 3, 7 \times 1\frac{1}{2}$	1/9	1/9	0/9	
Pooled results of groups 2 and 3				*5/20*	*3/20*	*0/20*	
4	adults	1 i.v.	2	3/21	0/21		

* Control.

spleen microsomes into very young females. This is in accord with earlier findings by Billingham and Silvers(10) who obtained tolerance in 20% of female C57 mice injected repeatedly a few days after birth with crude antigenic extracts obtained from lymphoid cells of syngeneic males. Moreover, the state of tolerance induced by spleen microsomes appears to be difficult to terminate by inoculation of normal lymphoid cells from syngeneic females; however, our observation is limited to only 4 animals. Billingham and Silvers also observed this in females rendered tolerant by inoculation at birth with viable syngeneic male lymphoid cells(10).

It is of note that Trakatellis *et al* reported a high degree of tolerance of syngeneic male skin grafts in female C57 mice conditioned by a single injection of microsomes, ribosomes or RNA obtained from syngeneic males, administered within 6 hours after birth(8). We attempted injections of microsomes at this age, but this attempt was abandoned because of the high degree of mortality.

Repeated i.v. inoculation of spleen microsomes to adult females prolonged survival times of male skin grafts on some of the recipients. The extended survival time of male skin grafts observed in this instance may be a function of the intravenous route of injection of the antigen. Previous studies in our laboratory indicated that administration of spleen microsomes by the intravenous route diminishes their capacity to induce maximum sensitization. Using a different system, Medawar observed prolongation of skin homograft survival in mice following intravenous injection of semisoluble transplantation antigens(11).

Failure to induce tolerance in adult mice stresses the fact that inoculation of the various antigenic preparations during some phase of immunological incompetence may be an essential prerequisite for induction of tolerance in this system. In addition, the results in this study support the concept that in this system persistence of an antigenic source may not be essential for maintenance of the state of tolerance. Finally, it can be concluded that microsomal fractions from male spleens are possessed of the Y chromosome antigens.

Summary. 1. Tolerance of syngeneic male skin grafts has been induced by repeated inoculation of male spleen microsomes to very young females. 2. Such treatment begun during adult life failed to induce tolerance but resulted in prolongation of graft survival in some of the treated females.

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