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BRIEF COMMUNICATIONS

PARTIAL SPECIFIC UNRESPONSIVENESS TO SECOND-SET ALLOGRAFTS FOLLOWING CHRONIC REJECTION OF FIRST-SET ALLOGRAFTS

According to the basic rules of transplantation immunity, allografting should provoke specific sensitization of the recipients. Sensitization manifests itself not only in the rejection of the allografted tissue or organ but also in the inevitable accelerated rejection of a second allograft having the same histocompatibility antigens.

This study presents experimental data suggesting that, under certain circumstances, the rejection of a first-set allograft may be accompanied by partial specific unresponsiveness (prolonged graft survival), instead of sensitization (accelerated rejection) to a second-set allograft. A short-term treatment of recipients with heterologous antithymocyte serum (ATS) resulting in long-term survival and modified (chronic) rejection of first-set allografts has been a requirement of producing such a partial specific unresponsiveness.

Allografting was performed between isogeneic mouse strains differing at strong histocompatibility antigens. A (H-2^a) and C57BL/10 (H-2^b) mice served as donors and CBA (H-2^k) male mice as recipients. Tail skin allografting was based on the method of Billingham and Medawar (*2*). Furthermore, preparation and immunosuppressive assay of rabbit antimouse ATS was done by a slight modification (15) of the method of Levey and Medawar (*10*).

Table 1 comprises the results. All those recipients which showed prolonged survival of first-set A allografts over 100 days following ATS treatment (2 × 0.5 ml s.c.) were selected and regrafted with a second-set A graft and/or a first-set C57BL/10 graft between the 115th and 130th days (group 1). No immunosuppressive treatment was given at the time of regrafting. At that time all primary grafts showed signs of either incomplete or complete chronic rejection as judged macroscopically by episodes of scaliness and scabbing, shrinkage of the graft, and gradual loss of hair, finally resulting in a smooth and shiny graft surface without any visible remnants of tail skin structure. Completion of chronic rejection generally requires 2–4 weeks.

In spite of the rejection of the first-set A grafts on ATS-treated mice, second-set A grafts on the same animals showed significantly longer survival than either second-set A grafts on non-treated recipients (groups 1a and 3) or primary A grafts on normal CBA recipients (groups 1a and 2). Unresponsiveness to second-set A grafts on ATS-pretreated animals, however, was limited, since none of the secondary grafts survived longer than 38 days. Survival time of third party C57BL/10 grafts on these mice was significantly shorter than that of the second-set A grafts (groups 1a and 1b), although some prolongation as compared to the mean survival of C57BL/10 grafts on normal CBA recipients (groups 1b and 4) was also observed.

One might argue that the H-2 barrier between H-2^a and H-2^k is much less than between H-2^b and H-2^k, – due to the fact that H-2^a is a recombinant haplotype (H-$2K^k$, H-$2D^d$) which shares the H-$2K$ locus and I region with H-2^k. Thus, the observation that the prolongation of third party C57BL/10 (H-2^b) grafts is less than that of the second-set A (H-2^a) on CBA (H-2^k) recipients would be accounted for by a difference in degrees of antigenic disparity in the face of a nonspecific suppressor mechanism. To rule out this possibility we started another set of experiments. The experimental conditions were exactly the same as described above (see Table 1) with the only difference that in this case C57BL/10 grafts served as first-set and second-set allografts while A grafts were used as third party grafts.

Table 2 shows that second-set C57BL/10 grafts survived significantly longer than A third-party grafts on CBA recipients conditioned with ATS pretreatment *plus* first-set C57BL/10 grafts (groups 1a and 1b). These results seem to indicate that the prolonged

TABLE 1. Survival times of second-set A and third-party C57BL/10 skin grafts on ATS-pretreated CBA mice chronically rejecting first-set A grafts over 100 days

Condition of first-set A grafts at regrafting	Survival times in days of (a) Second-set A grafts[b]	(b) Third-party C57BL/10 grafts[b]
Group 1[a]		
Incomplete rejection	10	13
	20	21
	21	ND[d]
	22	ND
	29	9
	29	ND
	ND	8
Complete rejection	15	22
	17	13
	28	16
	31	15
	32	16
	33	17
	38	20
	ND	15
	ND	13
Mean survival time (MST) (±SE)	*25.0* (2.26)	*15.2* (1.17)
Group 2[b, c]		
MST of second-set A grafts on 12 CBA mice	*7.9* (0.40)	
Group 3[c]		
MST of first-set A grafts on 32 CBA mice	*11.2* (0.26)	
Group 4[c]		
MST of first-set C57BL/10 grafts on 27 CBA mice	*11.3* (0.41)	

[a] Mice were given 2 × 0.5 ml ATS s.c. on days +2 and +5 after the first grafting.

[b] Grafted between the 115th and 130th days after the first grafting.

[c] Non-ATS-treated controls.

[d] ND, not done.

Significance of differences (Student's *t* test): group 1a + 3, $P < 0.001$; group 1a + 2, $P < 0.001$; group 1a + 1b, $P < 0.001$; group 1b + 4, $P < 0.001$.

survival of second-set allografts in conditioned mice may not be accounted for by a simple nonspecific suppressor mechanism. Furthermore, a significant although limited prolongation of third-party A grafts on conditioned CBA mice—as compared to A grafts on normal CBA mice—could be observed (groups 1b and 2).

We are unaware of any previous report on the occurrence of deficient graft reactivity across a strong histocompatibility barrier after graft rejection. Werder and Hardin (*16*) described prolonged survival of consecutive grafts after the rejection of primary grafts on nonimmunosuppressed mice with undetermined genetic background. Furthermore, Flaherty and Bennett (*6*) found that first skin grafts were often rejected while later ones were accepted on congenic mouse strains differing at loci determining H (*Ly-1*) and H (*T1a*) differentiation antigens. Billingham et al. (*1*) reported the return of "virgin" reactivity after termination of neonatal tolerance as judged by second-set grafting. Elkins et al. (*4*), Silvers (*14*), and Miyamoto and McCullagh (*11*) state that the termination of neonatal tolerance does not necessarily mean the return of immunological nor-

TABLE 2. Survival times of second-set C57BL/10 and third-party A skin grafts on ATS-pretreated CBA mice chronically rejecting first-set C57BL/10 grafts over 100 days

Condition of first-set C57BL/10 grafts at regrafting	Survival times in days of (a) Second-set C57BL/10 grafts	(b) Third party A grafts
Group 1		
Incomplete rejection	9	ND
	112	15
	>120[a]	14
Complete rejection	13	13
	20	ND
	27	ND
	41	17
	44	15
	73	17
	>120[a]	ND
Mean survival time (MST) (±SE)	>*57.9* (14.20)	*15.2* (0.65)
Group 2		
MST of first-set A grafts on 32 CBA mice	*11.2* (0.26)	

[a] Grafts still living at day 120.

Significance of differences (Student's *t* test): group 1a + 1b, $P < 0.05$; group 1b + 2, $P < 0.01$.

mality estimated by the intensity of MLC or graft-versus-host (GvH) reactivity. In these experiments, rejection of primary allografts on previously tolerant animals was taken as evidence of the termination of transplantation tolerance. Unresponsiveness to second-set grafts after the rejection or primary grafts was not demonstrated. In addition, neonatal tolerance should not be considered as an equivalent of suppressed immunoreactivity produced by ATS pretreatment in adult animals.

As regards the moderately prolonged survival of third-party grafts on conditioned animals, only speculations might be allowed at present. Weak histocompatibility antigens shared by A and C57BL/10 mice might be suspected as a cause of a limited degree of cross-tolerance (*7*). Another possibility is that public H-2 specificities may contribute to the prolonged survival of A and C57BL/10 grafts (*3*). However, the role of nonspecific factors cannot be excluded.

Furthermore, it should be mentioned that, according to clinical experiences, second kidney transplants are most successful when they are carried out to patients who have chronically rejected their first kidney grafts. In contrast, patients who acutely reject their first transplants take second grafts less well (*8*, *12*, R. L. Simmons, personal communication).

Our data may be interpreted as a special case in which the rejection of first-set allografts does not result either in the sensitization of the host or in the reversal to the virgin state of reactivity, but rather in a state of partial specific unresponsiveness as indicated by the fate of second-set allografts having the same histocompatibility antigens. The use of the term "partial specific unresponsiveness" seems to be justified on the basis of the following considerations: (1) in most cases, the state of unresponsiveness to second-set allografts is of relatively short duration (partial); (2) second-set allografts survive significantly longer than third-party allografts (specific); (3) we have at present no proper explanation for the experimental fact of prolonged survival of second-set allografts on ATS-pretreated recipients. Influence of serum-mediated blocking factors, failure of cellular response, and suppressor effect of T cells (*5*, *9*, *13*) seem to be the most likely explanations but other or combined mechanisms may also be involved. Till the elucidation of the mechanism of the prolonged survival of second-set allografts, the noncommittal term "unresponsiveness" is proposed.

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