Brief Definitive Report

CELLULAR BASIS OF TOLERANCE
IN NEONATALLY INDUCED MOUSE CHIMERAS*

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The mechanisms responsible for inducing and maintaining tolerance of foreign grafts remain to be elucidated. Among the possibilities, and they are not mutually exclusive, is that transplantation tolerance results from an active immune response which suppresses the capacity to reject the graft (1–5), and that appropriate exposure to foreign transplantation antigens can specifically eliminate those clones of cells which normally would mediate rejection (6, 7). Studies revealing specific unresponsiveness among populations of T cells from established chimeras in graft-vs.-host (GVH) (8), and mixed leukocyte culture (9, 10), reactions have been interpreted to support the clonal deletion hypothesis, particularly in situations where suppressor T cells and alloantibody formation could not be demonstrated (4, 9, 10). We now provide evidence that the induction and maintenance of transplantation tolerance as reflected by selective acceptance of a skin allograft may likewise depend upon clonal deletion.

Materials and Methods

The Principle of the Experiment. In a previous analysis (11) it was demonstrated that established A-strain skin grafts on CBA mice rendered tolerant at birth could be destroyed by the passive transfer of C3H anti-A lymphoid cells. Moreover, evidence was presented that these C3H cells also eliminated cell chimerism and that they too were rejected. Inasmuch as such treated tolerant mice rejected subsequent A-strain skin grafts, it was concluded that the destruction of these second grafts was mediated by immunologically competent cells of host genotype, i.e., cells which became competent as a result of the elimination of A-strain antigen. This raised the question whether the demonstrable host reactivity after recovery from tolerance was due to the maturation of new immunocompetent lymphocytes with specificity for the hitherto tolerated histocompatibility antigens, or, to the recovery of competence of cells which were previously "blocked" during the tenure of tolerance. Inasmuch as it seemed likely that the first alternative would require the presence of a thymus as a source of the new immunocompetent cells, whereas the second would not, we decided to repeat this analysis with tolerant subjects, some of which were thymectomized as young adults.

Mice. Male and female mice of the isogenic CBA/Ss (H-2^k), A/Ss (H-2^a), C3H/HeJ (H-2^k), and B10.A(4R) (H-2^{h-3S9}) strains as well as A/CBA F_1 hybrids were used.

Experimental Protocol. Tolerance was induced in CBA mice less than 18-h old by inoculation, via the orbital branch of the anterior facial vein, of 20 million spleen and lymph node cells from A/CBA male donors (12). All cell suspensions were prepared in Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) according to procedures described elsewhere (12), and were administered in a standard vol of 0.1 ml.

The putatively tolerant animals were either thymectomized (13), or sham-thymectomized when 4–5 wk of age and were challenged with A-strain skin grafts (12) when 7- to 9-wk old to confirm their tolerant status. After each tolerant or tolerant-thymectomized animal had maintained its A-strain graft in excellent condition, for at least 100 days, it was inoculated intraperitoneally (i.p.) with 150 million C3H lymphoid cells derived from the lymph nodes and spleens of C3H mice which had been sensitized against strain-A antigens. The sensitization procedure included challenging the donors bilaterally with A skin grafts followed, 10 days later, by a booster i.p. inoculation of 50 million A-strain spleen and lymph node cells. The lymphoid cells of these C3H mice were harvested 7 days later.

All originally tolerant CBA mice were challenged 50 days after receiving the C3H anti-A lymphoid cells with a second strain-A skin graft, and 25 days later with a C3H graft. Finally, 30 days after receiving this latter graft, all of the surviving tolerant-thymectomized animals received a B10.A(4R) skin graft as an additional means of assessing their immunologic competence.

Results and Discussion

As anticipated, all CBA mice tolerant of A-strain skin grafts rejected these grafts within 2 wk after receiving C3H anti-A cells. However, whereas all 5 of the nonthymectomized animals rapidly destroyed second A-strain grafts, only 4 of 10 of the thymectomized-tolerant mice rejected these grafts (Table I). Thymectomies were judged complete in these four mice by gross and histological examination. These rejections might have been mediated by T cells recovered from a blocked condition in animals which may or may not have been completely tolerant to begin with, or else by some mechanism not dependent upon T cells. More instructive are the mice which did not reject the second graft. In two thymectomized animals, which died of natural causes, the grafts were retained in excellent condition for 48 and 76 days, respectively, whereas in the other four the grafts remained in perfect condition for more than 100 days, after which the animals were killed.

The fact that six of the thymectomized CBA animals inoculated with C3H anti-A lymphoid cells subsequently accepted an A-strain graft indicates that they were probably competent, immunologically, to have eliminated the C3H cells. Nevertheless, it could still be argued that after these cells had been eliminated but before the second A graft was placed, these six adult thymectomized mice became sufficiently debilitated to accept the second A graft (14, 15). To test this possibility, as well as the chance that the C3H anti-A cells persisted and were responsible for destroying the second A-strain grafts on the other four thymectomized mice, both thymectomized and nonthymectomized animals were challenged with C3H skin, 25 days after receiving the second A graft. In all instances, except one, an animal which died 23 days after receiving the C3H graft, these grafts were rejected (Table I).

To further substantiate the fact that the thymectomized-tolerant mice which accepted A-strain grafts remained immunologically competent to other sets of histocompatibility antigens, four of them were challenged with H-2D-incompatible B10.A(4R) skin grafts a month after they received the C3H graft and more than 220 days after they had been thymectomized. All of these grafts were rejected (Table I).

It thus appears evident that, unlike the situation in intact mice where the elimination of chimerism has heretofore always resulted in the permanent abolition of neonatally induced tolerance (11), this is not the case in tolerant

TABLE I

Survival of Skin Grafts on Tolerant and Tolerant-Thymectomized CBA Mice

Animal	Survival of:			
	A graft after inoculation C3H anti-A cells	Second A graft	C3H graft	B10.A(4R) graft
	days	*days*	*days*	*days*
Tolerant-thymectomized				
♀1	13	>48*	>23	—
♀2	11	>100	11	11
♀3	8	10	13	—
♂4	13	>100	19	15
♂5	9	>76‡	18	—
♀6	10	>100	13	11
♀7	9	10	—§	—
♀8	10	10	13	—
♀9	9	10	11	—
♂10	10	>100	19	11
Tolerant				
♀11	9	11	11	—
♀12	9	12	11	—
♂13	10	12	11	—
♂14	10	10	11	—
♂15	10	10	11	—

* Animal died with A-strain graft in excellent condition and with C3H graft displaying 75% survival.
‡ Animal died with A-strain graft in excellent condition.
§ Animal died.

animals thymectomized as young adults. This observation lends support to the clonal-deletion hypothesis of tolerance (6).

It suggests that in the six acceptor mice there were few, if any, reversibly blocked mature T-cell clones which could recover competence to react with foreign strain-A histocompatibility antigens after adoptive abolition of chimerism. Rather these missing clones seem to recover only by maturation of a fresh set of T cells.

A similar conclusion was drawn from studies of GVH competence of rats in which neonatally induced tolerance had been adoptively terminated. In these studies direct and unequivocal evidence for specifically depleted clones of T cells was provided by experiments utilizing chromosome markers to identify the origins of T cells proliferating in response to specified sets of major histocompatibility complex alloantigens (8). The present work tends to support the conclusions drawn from the rat GVH study, and relates these conclusions to the effector mechanism of graft rejection in mice.

It could be argued that serum blocking factors persisted in the thymectomized but not in the intact mice and so accounted for acceptance of the second A grafts. We believe this unlikely in view of the evidence that serum blocking factors disappear when a state of chimerism is adoptively abrogated (16).

It might also be argued that suppressor T cells of host origin played a role in the acceptance of the second A grafts. This possibility seems remote since suppressor T cells are thought to disappear within a month after adult thymectomy in the mouse (17), and so should have been absent well before the time the second grafts were placed. These arguments of course do not bear one way or the other on the possibility that suppressor cells and alloantibodies may facilitate the induction of tolerance. Nevertheless, whatever the mechanism of tolerogene-

sis in the neonatal mouse, the process results in, and is revealed as, a deficit of specific clones from the normal T-cell repertoire.

Summary

In mice, thymectomized as young adults, neonatally induced tolerance persists in the putative absence of cell chimerism. This finding provides evidence that a selective deficiency of specific clones of lymphocytes exists in transplantation tolerance when induced under the conditions of these experiments.

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