MECHANISMS MAINTAINING ENHANCEMENT OF ALLOGRAFTS

I. Demonstration of a Specific Suppressor Cell

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The mechanisms that allow the long-term survival of major histocompatibility complex (MHC)[1]-immunologically enhanced organ allografts are poorly understood (1–5). In rodents, enhancement can be induced by treatment with either hyperimmune serum at the time of grafting or prior immunization with donor alloantigen. After a treatment rejection episode, many of these rats develop a state in which the immune system capacity of the host to develop a rejection response is specifically inhibited (1–5). Specific unresponsiveness to donor strain alloantigen in animals with established enhanced grafts is manifested by an inability to reject directly vascularized organ grafts from a second donor strain, but a normal capacity to reject third party grafts (3–6). Also, in rats with enhanced grafts, the rejection of donor strain but not third party skin grafts, is delayed and the length of the delay is inversely related to the time after grafting (7). This suggests that these animals have a specific inhibitory response that matures with time.

Although the induction of enhancement is antibody mediated, attempts to demonstrate humoral mediators during the maintenance phase of enhancement have been unsuccessful. After passive enhancement, grafts survive long after the injected antibody has been metabolized, and in recipients with actively enhanced grafts, the alloantibody titers wane after the first month (3, 8). Serum from rats with enhanced grafts will not enhance graft survival in a second host, and attempts to identify antiidiotype or blocking antibodies in the serum have mostly been unsuccessful (2, 9–12). Attempts to identify the cellular basis of this inhibitory response, which maintains prolonged graft survival, have thus far failed to demonstrate a suppressor cell mechanism. Hendry et al. (13) demonstrated a specific suppressor cell in thymus and spleen of rats during the induction phase of enhancement, but this effect, which could be transferred to nonimmunosuppressed rats, waned with time and was not demonstrated in the maintenance phase. Fabre and Morris (9) adoptively transferred spleen cells from rats bearing enhanced kidney allografts to normal rats grafted with kidney allografts, but they failed to transfer enhancement. Batchelor et al. (14) also failed to adoptively

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[1] *Abbreviations used in this paper:* ALG, antilymphocyte globulin; GVH, graft-vs.-host; LNC, lymph node cells; MHC major histocompatibility complex; MLC, mixed lymphocyte culture.

transfer unresponsiveness to partially immunosuppressed rats that had skin as the indicator graft. In all these systems, the adoptive host had a complement of lymphocytes adequate to effect rejection of the indicator graft. Thus, any suppressor cells would have had to overcome the alloreactivity of these normal lymphocytes.

Suppressor cells have been demonstrated in a wide variety of immune responses including some alloimmune responses (15–17). Dorsch and Roser (15) showed that in rats with transplantation tolerance induced in the neonatal period, recirculating T cells can adoptively transfer unresponsiveness to irradiated rats but not to normal rats. In animals with specific unresponsiveness to grafts, akin to enhancement but induced by nonspecific immunosuppression, such as antilymphocyte globulin (ALG) or cyclosporine treatment, suppressor T cells have also been demonstrated by adoptive transfer of spleen cells to irradiated hosts (16, 17).

To maximize the chance of identifying a suppressor mechanism in the maintenance phase of passively induced, enhanced heart grafts, we tested the capacity of cells from these animals to adoptively transfer unresponsiveness to irradiated heart-grafted hosts. In this model, adoptive transfer of normal cells will restore rejection (18). Thus, it was possible to examine whether cells from rats with long-surviving grafts had any specific changes in their capacity to effect rejection by adoptively transferring them to irradiated syngeneic recipients grafted with the original donor strain or third party strain hearts. A preliminary report of this work has been published (19).

Materials and Methods

Rats. PVG (RTI^c), DA (RTI^a), and Lew (RTI^l) strains and (DA × PVG)F_1 hybrids were bred and maintained at the Animal House, McMaster University, Hamilton, Ontario, Canada. The breeding pairs were kindly donated by Dr. A. Williams of the Sir William Dunn School of Pathology, Oxford, United Kingdom. AUG (RTI^c) strain were kindly donated by Dr. D. B. Wilson, Wistar Institute, Philadelphia.

Operative Procedures. Recipient rats, anesthetized by ether, were grafted with PVG hearts by end-to-side anastomosis of ascending aorta to abdominal aorta and pulmonary artery to inferior vena cava using 7-0 silk (Davis and Geck, Pearl River, NY), as previously described (20). Graft ischemic times ranged from 25 to 40 min and all grafts commenced to contract within 2 min of revascularization. Graft function was monitored daily and rejection was determined by the loss of palpable contractions and confirmed by the loss of electrocardiogram activity. Recipients were 250–300-g males and donors were 150–200-g females. Skin grafts were performed as described elsewhere (15).

Induction of Enhancement. Hyperimmune sera was made by applying a PVG skin graft to DA rats, then boosting three times with 5×10^7 PVG spleen cells given intraperitoneally every 14 d. These animals were bled out 7 d after the last booster injection and the blood was pooled and clotted. The serum, which had cytotoxic titers to PVG of 1:64–1:256, was frozen at 20°C until used. Enhancement was induced by giving 1 ml of hyperimmune serum intravenously to DA rats at the time of grafting with (DA × PVG) F_1 hybrid hearts. Rejection time was always delayed from a normal period of 6–10 d, but many rats did have a rejection crisis 10–21 d after transplant. In two-thirds of the rats, the grafts recovered from the rejection. Only those rats with hearts surviving >75 d were used as cell donors for the adoptive transfer experiments.

Cell Separation. Single-cell suspensions were prepared from spleen or pooled cervical and mesenteric lymph node cells (LNC) by gently squeezing them between the flat bottom of a 50-ml glass beaker and the floor of a petri dish. After washing twice at room

temperature in Dulbecco's phosphate-buffered saline containing calcium, magnesium, and bovine serum albumin (0.06%) (Armour Pharmaceutical Co. Ltd., Hampden Park, Eastbourne, Sussex, United Kingdom), preparations had a viability of 85–95% by trypan blue exclusion.

In Vitro Cell Preparation. The rosetting technique described by Parish and Hayward (21) was used. Briefly, goat anti-rat globulin (lot 4544; Cappel Laboratories, Cochranville, PA) was fixed to fresh sheep red blood cells using $CrCl_3$ and these cells formed rosettes with rat lymphocytes bearing surface immunoglobulin (sIg^+ cells). The nonrosetted lymphocytes were separated off on Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) Hypaque (Winthrop Laboratories, Aurora, Ontario, Canada), specific gravity, 1.098. These preparations contained <5% sIg^+ cells as assessed by staining with fluorescent goat anti-rat Ig (lot 8549A; Cappel Laboratories) using the labeling method described previously (16). More than 500 cells were examined with a Dialux microscope (E. Leitz, Inc., Rockleigh, NJ) fitted with Ploempak incident-light fluorescence and combined transmitted light phase contrast optics. 94–97% carried the peripheral T cell antigen Pta, A2, and B (22), which was detected by AUG anti-PVG antiserum, prepared according to the method of Howard and Scott (22) and used in two-stage immunofluorescence technique (18).

Recirculating T Cells. These were prepared as previously described (15, 18). Briefly, 1.5×10^9 spleen cells from DA rats with enhanced (DA × PVG)F_1 hybrid heart grafts were injected intravenously into intermediate hosts, which were DA rats that had been exposed to 1,000 rad whole body irradiation, had had their thoracic duct cannulated, and had been splenectomized. Cells were collected from the thoracic duct cannula of these rats for 36 h after their injection with spleen cells. These recirculating cells, 98% of which were Pta, A2, and B, and 2% sIg^+ cells, were tested in the adoptive transfer assay for their capacity to restore rejection.

Complement-dependent Cytotoxic Antibody Titers. These were performed essentially as described previously using PVG LNC as targets and guinea pig sera as complement (18). The endpoint was 50% killed cells.

Experimental Design. The adoptive transfer assay used has been described in detail (18). PVG or Lew hearts were grafted into DA recipients <24 h after both the donor and recipient rats had been exposed to 750 rad from a ^{60}Co source of a rate of 100–120 rad/min. Groups of rats grafted with PVG and Lew hearts were given intravenous injections of similar inocula of lymphoid cells. The speed of graft rejection was compared between these groups. Differences were analyzed using the Wilcoxon rank sum test and $P < 0.05$ was considered a significant difference. Results are expressed throughout as mean rejection time ± SD (in days).

Results

Capacity of LNC to Adoptively Restore Heart Graft Rejection in Irradiated Rats. In previous studies (18, 23), we have shown that LNC from normal rats will adoptively restore graft rejection in irradiated rats, and that the time taken to reject the graft is inversely related to the number of cells in the inoculum. A given inoculum has a similar restorative effect on PVG or Lew heart graft rejection (see Table I). This assay was used to detect any specific changes in alloreactivity of LNC from DA rats with enhanced (DA × PVG)F_1 hybrid heart grafts that had functioned for at least 75 d. Inocula of 1.5×10^8 and 5×10^7 LNC cells from these rats restored PVG graft rejection, but with a small and significant delay compared with the rejection time in rats restored with similar numbers of normal LNC. 5×10^7 LNC from rats with enhanced grafts effected Lew graft rejection at the same rate as normal LNC. With 2×10^7 LNC, four of six PVG grafts were not rejected in 100 d, but Lew grafts were all rejected and at the same rate as occurred with 2×10^7 normal LNC. Control rats not restored with cells did not reject in <45 d.

TABLE I

Heart Graft Survival Time in Irradiated DA Rats Restored with LNC for Either DA With Enhanced (DA × PVG) F_1 PVG Heart Grafts or Normal DA Rats

Restorative inocula		Rejection time of PVG grafts		Significance			Rejection time of Lew grafts	
Number	Source	Days	Mean ± SD				Days	Mean ± SD
1.5×10^8	Enhanced	10, 12, 14, 15, 16	13.4 ± 2.4	$P < 0.05$	—	—	ND*	
1.5×10^8	Normal	8(2), 9(2), 10, 11(2), 12	9.75 ± 1.5		NSD‡		8, 9, 10, 12	9.75 ± 1.7
5×10^7	Enhanced	11, 13, 15, 17, 18	14.8 ± 2.9	$P < 0.01$		NSD	9, 11, 13(2), 15	12.2 ± 2.3
5×10^7	Normal	9(2), 10(2), 11(3)	10.1 ± 0.9		NSD		9, 10, 12, 13, 14, 15, 16	12.6 ± 2.8
2×10^7	Enhanced	25, 28, >80(4)	>80	$P < 0.05$	$P < 0.05$		16, 19, 23(2), 25	21.2 ± 3.6
2×10^7	Normal	12, 19, 23, 25	25.4 ± 4.5					
—	—	46, 54, 63, 75, 92	66 ± 18.1		NSD		62, 67, 69, 74, 79, >100(2)	>79

* Not determined.
‡ No significant difference.

TABLE II

Heart Graft Survival Time in Irradiated DA Rats Restored With Spleen Cells from Either DA With Enhanced (DA × PVG) F_1 Heart Grafts or Normal DA Rats

Restorative inocula		Rejection time of PVG grafts		Significance		Rejection time of Lew grafts	
Number of LNC	Source	Days	Mean ± SD			Days	Mean ± SD
1.5×10^8	Enhanced	40, 49, >100(4)	>100		<0.01	11, 14, 16, 18, 20	15.8 ± 3.5
1.5×10^8	Normal	12, 14, 16(3), 18(2), 20	16.3 ± 2.5	<0.001	—	ND	
7.5×10^7	Enhanced T	15, 21, 25, >100(4)	>100	<0.05	<0.01	10, 12, 13, 15, 16	13.2 ± 2.4
7.5×10^7	Normal T	13, 15, 17, 19, 21	14.6 ± 2.7			ND	

As over 5×10^8 LNC can be obtained from rats with enhanced grafts, these experiments show that these rats have a surplus of cells with the capacity to mediate PVG rejection. The small reduction in the capacity of LNC from rats with enhanced (DA × PVG)F_1 grafts to effect PVG rejection was shown to be specific by the finding that they restored Lew rejection to the same rate as normal LNC (Table I).

These results suggest that either there was a relative deficiency in the proportion of alloreactive cells to PVG compared with Lew, which is best manifested with the smaller inocula of 2×10^7 LNC, or that some specific inhibitory mechanisms may have overridden the effector cells in the smaller inocula.

Capacity of Spleen Cells to Adoptively Restore Heart Graft Rejection in Irradiated Rats. Spleen cells from rats with long-surviving grafts showed a marked, specific loss in capacity to restore PVG graft rejection (Table II). 1.5×10^8 spleen cells failed to restore rejection in the time a similar number of normal spleen cells can. In four of six rats, PVG graft rejection was not effected, and the other two did not reject their grafts until >40 d. Loss of reactivity was specific in that 1.5×10^8 spleen cells from rats with enhanced grafts effected third party graft rejection in <20 d. None of the six rats restored with smaller inocula (5×10^7)

TABLE III

Graft Survival Time of Heart Grafts in Irradiated DA Rats Restored with Recirculating T Cells Obtained from Spleen Cells of DA Rats with Enhanced (DA × PVG) F_1 Grafts

Number of cells	Donor strain	Rejection time (days)	Mean ± SD	Significance
1.5×10^7	PVG	11, 13, 15, 17(2)	14.6 ± 2.6	NSD
1.5×10^7	Lew	10, 11(2), 15(2)	12.4 ± 2.4	

rejected PVG grafts within 100 d. Larger inocula (4×10^8) also did not effect rejection when tested in two rats.

To examine whether the failure of spleen cells to adoptively restore rejection was due to a loss of alloreactivity of the T cell fraction of the spleen, we depleted the spleen cells of Ig^+ cells using a rosetting technique. Cell fractions prepared in this way were 95–99% Pta^+ and 2–5% sIg^+, compared with 45–50% Pta^+ and 50–55% sIg^+ in unfractionated spleens. 7.5×10^7 T cells, which is equivalent to the number of T cells in 1.5×10^8 unfractionated spleen cells, were tested in the adoptive transfer assay. These T cell fractions of spleen also showed a specific lack of capacity to adoptively restore PVG graft rejection, but had a normal capacity to restore Lew graft rejection (Table II). Cell for cell, normal spleen cells are less effective than LNC at restoring graft rejection, in that 1.5×10^5 spleen cells only restore rejection to <20 d and 1.5×10^8 LNC to <10 d. We have previously shown (18) that in spleen and LNC, the cells that adoptively restore rejection are T cells of the recirculating pool and that the difference in capacity of these cell populations to restore rejection is related to the smaller proportion of recirculating T cells in the spleen.

Capacity of Recirculating T Cells to Adoptively Restore Heart Graft Rejection in Irradiated Rats. In rats with neonatally induced transplantation tolerance, the recirculating T cells contain suppressor cells that adoptively transfer tolerance to irradiated hosts (15). To test whether the spleens of rats with enhanced grafts were depleted of recirculating alloreactive T cells, or contained a recirculating suppressor T cell similar to that found in neonatal tolerance, recirculating T cells were prepared as described in Materials and Methods and these inocula were essentially nearly all T cells (98% Pta^+, 2% sIg^+). There was no difference in their capacity to adoptively restore both PVG and Lew graft rejection (Table III). Thus, spleen from rats with enhanced grafts do have recirculating T cells with a normal capacity to adoptively restore graft rejection.

These studies suggest that within the cell fraction of spleen, there may be a suppressor population that inhibits the capacity of the recirculating T cells present in the spleen to effect the rejection response.

Capacity of Spleen Cells to Inhibit Normal LNC Capacity to Adoptively Restore Heart Graft Rejection in Irradiated Rats. Spleen cells and splenic T cells were tested for their capacity to inhibit the restoration of graft rejection by normal LNC. As the ratio of suppressor to effector cells may be critical, an inoculum of normal LNC (3×10^7), which only partially restored rejection to normal, was mixed with 1.5×10^8 spleen cells from rats with enhanced grafts. The spleen cells inhibited the capacity of the normal LNC to effect rejection (Table IV).

The capacity of T cells to mediate this effect was also tested. Inocula of 7.5 ×

TABLE IV

PVG Heart Graft Survival Times in Irradiated DA Rats Restored with Spleen Cells or In Vitro-Separated T Cell Fraction of Spleen Cells from DA Rats with Enhanced (DA × PVG) F_1 Heart Grafts and Normal LNC

Number of cells from		Rejection time (days)	Mean ± SD	Significance
Enhanced spleen	Normal LNC			
1.5×10^8	—	55, >100(5)	>100	NSD
1.5×10^8	3×10^7	41, 56, >100(4)	>100	NSD
7.5×10^7 T	—	26, 32, >100(4)	>100	NSD
7.5×10^7 T	3×10^7	20, 23, 27, 30, 34(2)	28.0 ± 5.8	$P < 0.001$
—	3×10^7	14, 16(4), 18, 19(4)	17.2 ± 1.8	$P < 0.001$
—	—	46, 51, 56, 61, 66, 80	60.0 ± 12.1	

10^7 T cells prepared by rosetting also significantly delayed the capacity of the normal LNC to restore rejection but never established a state of enhancement, as all grafts were eventually rejected (Table IV). This suggests that the T cells in spleen only partially mediate suppression and that other mechanisms, including antibody-mediated responses, may contribute to mediation.

Cytotoxic Antibody Response in Adoptively Restored Rats. Rats restored with 1.5×10^8 spleen cells from enhanced rats and 3×10^7 normal LNC were tested for cytotoxic antibodies 7, 14, and 28 d after their restoration. No detectable antibody was found. Control irradiated DA rats restored with 1.5×10^8 normal spleen cells had antibody titers of 1:16 to 1:64 at 7 d, and 1:32 to 1:128 at 14 d. Control hyperimmune sera always had a cytotoxicity of 1:64 to 1:256. The target for these assays was PVG LNC, 40% of which are B cells. In none of the assays of sera from rats adoptively restored with cells from rats with enhanced grafts was the cytotoxicity of the LNC above background. An antibody response to class II MHC antigens detectable by cytotoxicity to B cells was also not identified. Thus, the capacity of spleen cells to inhibit the normal LNC capacity to restore rejection could not be ascribed to a transfer of complement-dependent cytotoxic antibody response to conventional class I or class II MHC antigens.

Immune Status of Rats Adoptively Restored with Spleen Cells from Rats With Enhanced Grafts. Four DA rats that had been irradiated and restored with spleen cells from DA rats with enhanced grafts and had had PVG heart grafts surviving for over 75 d were tested for their alloreactivity by skin-grafting them with PVG and Lew grafts. PVG skin grafts were not rejected at the first-set rate, as one rejected the graft in 13 d, one in 23 d, and the other two retained their grafts for over 80 d. The first-set rejection rate for PVG on normal DA rats is 8–10 d. In contrast, Lew skin was rejected in 8, 9, and 10 (2) d, respectively, which is at the first-set rate (8–11 d). All four rats retained their PVG heart graft and there was no detectable rejection crisis at the time the PVG skin was rejected. In another group of rats adoptively restored with spleen cells from rats with enhanced grafts, where PVG grafts surviving >75 d were challenged with 2×10^8 normal LNC, these cells also failed to effect rejection and all allografts continued to function for another 50 d. These experiments suggest that the adoptively restored irradiated hosts develop a form of specific unresponsiveness

similar to that seen in normal rats in which graft survival has been enhanced with antisera.

Discussion

These experiments showed that rats with passively enhanced, long-surviving cardiac allografts had sufficient recirculating T cells with reactivity toward the graft alloantigens to initiate and effect rejection of donor strain heart grafts. These findings are consistent with the fact that when exposed to donor strain antigens, cells from animals with enhanced grafts can proliferate in mixed lymphocyte culture (MLC) and graft-vs.-host (GVH) reactions and generate cytotoxic T cells with the same vigor as normal lymphocytes (2, 3, 7, 24–26).

The failure of these alloreactive cells to effect rejection of the enhanced graft was explained by the demonstration that splenic T cells from these rats can inhibit the capacity of normal lymphocytes to restore graft rejection in irradiated hosts. Irradiated rats to which enhancement was transferred were also found to have a state of specific unresponsiveness that was not due to reinduction of enhancement by transfer of a detectable cytotoxic alloantibody response. However, these studies did not exclude a possible role for other antibody responses, such as antiidiotype or non–complement-fixing alloantibodies, which could block alloantigen or opsonize alloantigen-reactive cells, as suggested by Hutchinson (27). Even though the removal of B cells in vitro did not remove the capacity of spleen cells to adoptively transfer suppression, the possibility cannot be excluded that the small (2–5%) contamination with B cells in the in vitro separated T cell population did not mediate the inhibition. The failure of sera from rats with long-surviving enhanced grafts to induce enhancement of kidney grafts in normal recipients (2, 3) or in heart graft-irradiated recipients restored with small numbers (3×10^7) of normal LNC, argues against a role for antibody (B. M. Hall, unpublished observation). Furthermore, antiidiotypic antibodies can be demonstrated in the induction but not the maintenance phase of enhancement (2, 12) and attempts to demonstrate blocking antibody in vitro have also usually failed (11).

As suppressor T cells in rats with enhanced grafts are not recirculating cells, they are quite different from those in rats with neonatally induced transplant tolerance, which can be adoptively transferred with recirculating T cells. The suppressor cell in rats with enhanced grafts has several other characteristics which suggest that it operates by a different mechanism and against a different target than the suppressor T cell demonstrated in neonatally induced transplant tolerance. The suppressor T cell in neonatal tolerance, unlike that in rats with enhanced grafts, leads to effective specific deletion of clones of cells reactive to donor antigens in MLC and GVH, as well as those with the capacity to adoptively restore rejection of donor strain grafts in irradiated hosts (15). The suppressor cells in the spleen of rats with enhanced grafts were shown to suppress the allograft activity of normal LNC populations. However, the suppressor cells from transplant-tolerant animals, although capable of specifically suppressing the regeneration of alloantigen-sensitive cells in irradiated hosts, are not capable of suppressing the alloreactivity of mature peripheral lymphocytes (15). Finally, the suppression in neonatal tolerance is dependent upon the maintenance of the

chimeric state in these animals, while chimerism is not a feature of specific unresponsiveness induced in adult animals, including rats with passively enhanced grafts (15, 17).

As our experiments suggested that suppression could only be demonstrated in circumstances where the number of normal alloreactive cells was the minimum required to effect rejection, it was not surprising that previous attempts to transfer suppression were not successful, as they had transferred spleen cells to animals that had sufficient numbers of their own lymphocytes to effect rejection of the indicator graft (9, 13, 14). Batchelor et al. (28–30) have suggested that maintenance of the suppressor state in enhancement is dependent upon a reduction in the number of donor strain dendritic cells within the graft. However, this explanation does not account for the fact that the application of donor strain skin or organ grafts or the injection of donor strain dendritic cells to animals bearing enhanced grafts does not induce a rejection response against the long-surviving graft. In our experiments, the adoptive host was transplanted with a graft that was immunogenic enough to stimulate as few as 2×10^7 normal LNC to restore rejection. Thus, we surmised that reduced immunogenicity of the graft in the adoptive host did not contribute to the transfer of enhancement. Combining our findings with those of Batchelor et al., it could be concluded that adoptive transfer of suppression in animals with enhanced grafts can only be demonstrated when the generation of a rejection against the graft is limited either by the number of alloreactive cells available to mediate rejection or by a markedly reduced immunogenicity of the graft.

The specific unresponsive state seen in animals with enhanced grafts is similar in many respects to that seen in animals after treatment with short courses of nonspecific immunosuppressives such as ALG (31) or cyclosporin A (17), or by active enhancement with donor blood (32) or donor liver extract, *Bordetella pertussis* vaccine, and ALG (16). In all these models, a suppressor T cell has been demonstrated in the spleen that has features in common with the suppressor cell demonstrated by our experiments.

In this report, the results suggest that active suppression, in which T cells play an important role, is a feature of the immune state in rats that have had prolonged allograft survival induced by passive enhancement. The mechanism whereby suppressor T cells operate to prevent the activation of recirculating alloreactive T cells in rats with enhanced grafts is not known. In particular, it remains to be answered whether this suppression is directed at helper/inducer T cell response, thus inhibiting delayed-type hypersensitivity–mediated rejection as well as help for alloantibody and cytotoxic T cell responses, or only against cytotoxic T effector cells (33), and whether suppression operates against responses to MHC antigens and minor histocompatibility antigens or only against MHC antigens; and the identity of the precise subpopulation of T cells mediating this suppression remains to be determined.

Summary

DA rats treated with hyperimmune anti-PVG serum and grafted with (DA × PVG)F_1 heart grafts in which graft survival was prolonged for >75 d were used to examine the cellular mechanisms that maintain the state of specific unrespon-

siveness found in these animals. The capacity of lymphocytes from these animals to effect or inhibit graft rejection on adoptive transfer to irradiated heart-grafted hosts was tested. Spleen cell populations and the T cell subpopulation separated from spleen cells in vitro failed to restore rejection of PVG heart grafts in irradiated DA recipients but restored third party Lew graft rejection. Whole spleen cells had the capacity to suppress the ability of normal DA LNC to cause graft rejection, but T cells from spleen only delayed the restoration of rejection. LNC and recirculating T cells from rats with enhanced grafts adoptively restored PVG rejection, however. These studies show that the state of specific unresponsiveness that follows the induction of passive enhancement is dependent in part upon active suppression, which is induced or mediated by T lymphocytes. The recirculating pool of lymphocytes in these animals is not depleted of specific alloreactive cells with the capacity to initiate and effect rejection. Thus, these animals' unresponsiveness is not like that found in transplantation tolerance induced in neonatal rats, but is, in part, due to a suppressor response that can inhibit normal alloreactive cells' capacity to initiate and effect rejection.

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