

SPECIFIC UNRESPONSIVENESS IN RATS WITH
PROLONGED CARDIAC ALLOGRAFT SURVIVAL AFTER
TREATMENT WITH CYCLOSPORINE

Mediation of Specific Suppression by T Helper/Inducer Cells

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A variety of treatments at the time of transplantation may induce rodents to accept major histocompatibility complex-incompatible organ allografts without further immunosuppression (1-7). Passively administered anti-donor strain hyperimmune globulin, active immunization with donor strain blood or lymphoid cells (1, 2), or short courses of either cyclosporine (CY)¹ (4, 5), or antilymphocyte globulin can all lead to indefinite graft survival in rats (6, 7). Following each of these treatments, a proportion of rats develop a state in which the immune system is incapable of mounting a rejection response against donor strain organ allografts, but retains the capacity to reject third-party allografts (1, 5, 8). In all cases, the state of unresponsiveness is not established for at least 7 wk after transplantation, and becomes progressively more marked with time, to the extent that a large proportion of the rats in which organ graft survival has been prolonged by one of the above measures will eventually accept donor strain skin grafts (1, 5, 8). Although humoral responses play an important role in the induction of many of these forms of unresponsiveness, their role in long-term maintenance of unresponsiveness has never been conclusively established (9-11). On the other hand, several groups have shown (8, 11-16) that unresponsiveness is actively mediated by host lymphoid cells, and that T cells contribute to this inhibitory response.

It is now recognized that there are several effector mechanisms with the potential to mediate graft rejection. Adoptive transfer experiments have demonstrated that both helper/inducer and cytotoxic/suppressor subsets of T cells can restore the capacity of immunosuppressed hosts to effect rejection (17-20). As far as cell populations from unsensitized animals are concerned, it is evident that the helper/inducer T cell subset restores rejection to irradiated hosts when injected either alone or with cytotoxic cells. When injected alone, however, neither the cytotoxic T cell subset nor B cells have the capacity to effect rejection (18, 20). Adoptive transfer systems have also been used to establish that cells from rats with long-surviving grafts do not restore rejection of donor strain

¹ Abbreviations used in this paper: CY, cyclosporine; LNC, lymph node cell; W/F, Wistar-Furth.

hearts in adoptive hosts, and furthermore, at least in the case of cells from enhanced or CY-treated donors, can inhibit the capacity of simultaneously transferred normal lymphocytes to do so (12–14, 16).

In this study, we examined the capacity of separated populations of helper/inducer or cytotoxic/suppressor subclasses of T cells and of B cells from CY-treated DA rats with long-surviving PVG heart grafts either to effect rejection or to transfer unresponsiveness in the adoptive allograft assay.

Materials and Methods

Rats. Rats of the DA (RT1^a), PVG (RT1^c), and Wistar-Furth (W/F) (RT1^u) strains were bred in the Blackburn Animal House, and maintained in the Pathology Animal House at the University of Sydney.

Operative Procedures. DA rats, anesthetized with ether, were grafted with PVG or W/F hearts by end-to-side anastomosis of the ascending aorta to the abdominal aorta and the pulmonary artery to the inferior vena cava, as described elsewhere (21). Donors were 150–200-g female rats, and recipients were 250–300-g male rats.

Graft ischemia times ranged from 25–40 min, and all grafts commenced to contract within 2 min of the restoration of blood supply. Graft function was monitored daily, and rejection was determined by loss of palpable contraction and confirmed by loss of electrocardiogram activity. On the day electrocardiogram activity was lost, an autopsy was performed and graft tissue was obtained for histological confirmation of rejection.

Cell Preparations. Single-cell suspensions from spleen and lymph node cells (LNC), were prepared as described elsewhere (22). Subpopulations of lymphocytes were identified by the mouse monoclonal antibodies W3/13 (pan-T), W3/25 (T helper/inducer), and MRC OX8 (T cytotoxic/suppressor) (Sera Laboratories, Oxford, United Kingdom), using an indirect immunofluorescence technique described elsewhere (18). Ig⁺ cells were identified, using fluorescein isothiocyanate-labeled rabbit anti-rat Ig (DAKO, Copenhagen, Denmark) (22).

Cell Separation. Full details of the cell separation techniques have previously been described (18). Ig⁺ cells were removed from whole lymphocyte populations by incubation of the cell preparation on plastic petri dishes (Lab Tek; Miles Laboratories, Inc., Naperville, IL) coated with rabbit anti-rat Ig (DAKO). The resultant cell preparations were 99% W3/13⁺ and <1% Ig⁺; 70–80% of the W3/13⁺ cells in the original preparation were recovered.

T cell subpopulations were separated by binding either MRC OX8 or W3/25 to the cells before panning on plates coated with both rabbit anti-mouse Ig (DAKO) and rabbit anti-rat Ig. The nonadherent population from MRC OX8-bound cell populations was 95–97% W3/25⁺, 1–2% Ig⁺, and 1–2% MRC OX8⁺, that from W3/25-bound cell populations was 90–95% MRC OX8⁺, 2–5% Ig⁺, and 1–5% W3/25⁺.

B cells were prepared by binding W3/13 to cells before panning on plates coated with rabbit anti-mouse Ig that had been blocked for anti-rat Ig activity by preincubation with normal DA rat serum. Nonadherent populations were 90–98% Ig⁺ and 5–10% W3/13⁺.

Treatment of Rats to Induce Long-term Graft Survival. DA rats were grafted with PVG hearts and given CY (a gift of Sandoz, Ltd., North Ryde, Australia) at a dose of 10 mg/kg body weight/day. CY, supplied as a powder, was diluted in olive oil, mixed overnight with a magnetic stirrer, then stored at 4°C. The appropriate dose was pipetted into the pharynx of lightly anesthetized animals. Grafted rats were given CY immediately postoperatively, and then once daily for 10 d. Grafts in treated animals survived for >100 d, compared to a survival time of 6–7 d in untreated controls. Cells from rats with PVG grafts surviving longer than 75 d were used in these studies.

Experimental Design. Donor and recipient rats for adoptive transfer experiments were irradiated from a ⁶⁰Co source at 150 rad/min to a total dose of 900 rad as described before (22), and within 24 h, the DA recipients were grafted with hearts from PVG or W/F donors. Irradiated DA recipients given no cells do not reject PVG or W/F hearts

for at least 50 d, and many retain their grafts indefinitely (>150 d). The capacity to reject a heart graft from either strain can be restored to irradiated recipients by the adoptive transfer of normal syngeneic LNC or spleen cells. Animals restored with equal numbers of normal cells reject PVG and W/F hearts with a similar tempo. Rejection of heart grafts after adoptive transfer of 1.5×10^8 LNC occurs at 9.3 ± 0.6 d for five subjects given PVG hearts, and at 9.7 ± 0.9 in five other rats given W/F hearts; after 3×10^7 LNC were transferred, rejection occurred at 15.2 ± 3.9 d (five rats), and at 17.0 ± 4.6 d (six rats) for PVG and W/F transplants, respectively. As normal cells display equal reactivity toward PVG and W/F grafts, this assay is ideal for the detection of a specific loss of alloreactivity in a given cell population. In addition, because the relationship between the number of cells used to restore rejection and the rejection time is quantitative, the assay reflects the extent of the loss of alloreactivity in a cell population.

Statistics. Differences in rejection time between groups of rats restored with equivalent inocula were analyzed using the Wilcoxon rank sum test. A *p* value of <0.05 was considered to reflect a significant difference.

Results

The starting point for these experiments was the observation that LNC and spleen cells, or their T cell fraction, from CY-treated DA rats with PVG heart grafts that had been accepted for >75 d failed to restore PVG heart graft rejection, but effected third-party (W/F) rejection when adoptively transferred to irradiated DA rats (Table I). In the few recipients in which cells from CY-treated rats did effect rejection, it was with a slower tempo than in control rats given an equivalent number of normal DA cells. The time course of rejection of W/F grafts in animals restored with cells from CY-treated rats with PVG grafts was not impaired, nor was it different from that achieved with normal cells.

When cells from DA rats with long-surviving grafts were mixed with normal LNC and adoptively transferred to irradiated grafted recipients, it was clearly shown that the former cell population did not merely lack reactivity towards

TABLE I
Survival of PVG and W/F Hearts in Irradiated DA Rats Restored with Cells from CY-treated Rats with Long-Surviving PVG Allografts

Restorative inocula			Rejection time					
Normal DA LNC	CY-treated DA LNC and spleen cells*		PVG hearts			W/F hearts		
	Number of cells	Fraction	Animals surviving on days (N) [‡]	Median	<i>p</i> [§]	Animals surviving on days (N) [‡]	Median	<i>p</i> [§]
	10^8	—	8, 9, 13 (3)	13		8, 10, 11, 13	10.5	NSD
	—	1.5×10^8	25, 26, >100 (6)	>100	<0.005	12, 14, 15, 21	14.5	<0.01
	3×10^7	1.5×10^8	20, 24, 26, >100 (5)	>100	NSD	13, 14, 15, 23	14.5	<0.01
	3×10^7	—	13 (3), 15, 22, 28	15	<0.02	14 (2), 15 (2), 18, 26	15	NSD
	3×10^7	7.5×10^7	10, 19, >100 (3)	>100	<0.05	NT		
	—	7.5×10^7	>100 (7)	>100	NSD	NT		
	—	—	40, 53, 68, >100 (5)	>100	NSD	>100 (4)	>100	NSD

* DA rats, previously treated with CY with PVG grafts surviving >75 d.

[‡] Numbers in parentheses are numbers of individuals surviving to that day. NT, not tested.

[§] Significance determined by Wilcoxon rank sum test. NSD, not significantly different.

specific antigen, but had the capacity, specifically, to inhibit the reactivity of normal cell populations (Table I). The identity of the inhibitory cell was sought in experiments in which lymphocyte subsets were separately assayed for their capacity to suppress normal cells.

Role of Helper/Inducer (W3/25⁺) Cells in Adoptive Transfer of Unresponsiveness. In a large number of adoptive transfer experiments, the W3/25⁺ cell has been shown to be the principal cell required for the restoration of graft rejection in T cell-depleted animals. Graft rejection can be adoptively restored with small numbers of W3/25⁺ cells, whereas even large numbers of MRC OX8⁺ cells from normal DA rats do not effect rejection (Table II). In fact, as few as 10⁶ W3/25⁺ cells can restore rejection in <20 d, and are as effective as 20 times more W3/25⁺ cells. These studies suggest that only a very few alloreactive W3/25⁺ cells are required to initiate the rejection response in the irradiated adoptive host. Tests of large numbers of W3/25⁺ cells from CY-treated rats with long-surviving grafts all failed to restore rejection.

Unfractionated LN and spleen cells contain 40% W3/25⁺ cells, therefore, inocula of 6×10^7 W3/25⁺, which represents the number present in 1.5×10^8 unfractionated cells, were used in experiments to test the role of W3/25⁺ cells in the adoptive transfer of unresponsiveness. 6×10^7 W3/25⁺ cells from normal DA donors restored both PVG and W/F rejection to the tempo achieved with 1.5×10^8 whole LNC. In contrast, 6×10^7 W3/25⁺ cells from DA rats with long-surviving grafts effected rejection of PVG grafts in only two of eight adoptive hosts, but effected rejection of all W/F grafts with a tempo similar to that achieved by the same number of normal W3/25⁺ cells (Table III). Thus, an inoculum of W3/25⁺ cells from rats with long-surviving grafts, with over 20

TABLE II
Survival of PVG Grafts in Irradiated DA Rats after Adoptive Restoration with W3/25⁺ or MRC OX8⁺ Cells Separated from Normal DA LNC

Restorative inocula			Rejection time		
Number of LNC-equivalents*	Number of cells	Subset	Day [†]	Median	p [§]
2×10^8	7.5×10^7	W3/25 ⁺	10, 12, 14 (2), 20, 24	14	
5×10^7	2×10^7	W3/25 ⁺	14 (2), 15 (2), 18, 20	15	NSD
10^7	5×10^6	W3/25 ⁺	11 (2), 12 (5), 14, 33	12	NSD
2.5×10^6	10^6	W3/25 ⁺	7, 9, 13, 14, 18, 27	13.5	NSD
2×10^8	3.3×10^7	MRC OX8 ⁺	>100 (4)	>100	<0.05
5×10^7	7×10^6	MRC OX8 ⁺	90, >100 (5)	>100	NSD

* Doses of T cell subsets were given in numbers equivalent to that found in an inoculum of whole LNC, on the basis that 37–43% are W3/25⁺ and 12–17% MRC OX8⁺.

† Numbers in parentheses are numbers of individuals.

§ Significance determined by Wilcoxon rank sum test. NSD, not significantly different.

TABLE III
Survival of PVG and W/F Heart Grafts in Irradiated DA Rats Restored with W3/25⁺ Cells from Either Normal or CY-treated DA Rats with Long-surviving PVG Grafts

Restorative inocula		Rejection time					
Source of W3/25 ⁺ cells (6 × 10 ⁷ cells used)	3 × 10 ⁷ normal LNC added	PVG hearts			W/F hearts		
		Animals surviving on day (N)*	Median	p [‡]	Animals surviving on day (N)*	Median	p [‡]
Normal	—	10, 12, 14 (2), 20, 24	14		10, 11 (2), 15, 20	11	
DA	—	18, 29, >100 (8)	>100	<0.01	13, 15, 17 (2), 18, 23	17	NSD
CY Rx	—	8, 12 (2), 40, >100 (4)	>40	NSD	NT		
DA [§]	+	11 (4), 15, 18	11	<0.05	NT		
CY Rx	+						
DA [§]	+						

* Numbers in parentheses are numbers of individuals. NT, not tested.

[‡] Significance determined by Wilcoxon rank sum test. NSD, not significantly different.

[§] DA rats previously treated with CY and bearing long-surviving PVG grafts.

times the number of cells required to restore rejection, showed a marked specific loss of alloreactivity.

We next examined whether the apparently unreactive W3/25⁺ cell population could actively inhibit the alloreactivity of normal cells. 6 × 10⁷ W3/25⁺ cells from DA rats with long-surviving grafts were mixed with 3 × 10⁷ normal LNC and adoptively transferred to irradiated DA rats with PVG heart grafts (Table III). Three of eight rats rejected their grafts within 12 d, one rat rejected its graft in 40 d, and four retained their grafts indefinitely (Table III). Control rats given 3 × 10⁷ normal LNC alone all rejected their grafts within 18 d. These results suggest that, in the experimental group, the added W3/25⁺ cells had inhibited the allograft responsiveness of the normal cells.

Because normal LNC contain both helper/inducer and cytotoxic T cells, and B cells, it is possible that the W3/25⁺ cells from the DA rats with long-surviving grafts had stimulated either the B cells or the cytotoxic/suppressor cells in the admixed normal population, and that these had in turn mediated an inhibitory response. Therefore, experiments were done in which W3/25⁺ cells from rats with long-surviving grafts were adoptively transferred with only the W3/25⁺ cell population separated from normal LNC (Table IV). Four of five irradiated DA hosts given 4 × 10⁷ W3/25⁺ cells from rats with long-surviving grafts and normal 5 × 10⁶ W3/25⁺ cells did not reject their grafts. 2 × 10⁷ W3/25⁺ cells also impaired the restorative capacity of 5 × 10⁶ normal W3/25⁺ cells. This suggests that the inhibitory effect is mediated by the W3/25⁺ cells themselves, and is not a consequence of the induction of an inhibitory response mediated by other cell populations in the adoptively transferred normal LNC population.

Role of MRC OX8⁺ Cells in Adoptive Transfer of Unresponsiveness. It has already been well established by others (17) and by us (Table II), that the cytotoxic/suppressor MRC OX8⁺ population from normal rats does not restore the graft rejection response to irradiated hosts, thus MRC OX8⁺ cells from CY-treated rats were tested only for their inhibitory capacity. 2 × 10⁷ MRC OX8⁺ cells from rats with long-surviving grafts (the number present in 1.5 × 10⁸ unfractionated

TABLE IV
Survival of PVG Hearts in Irradiated DA Rats Restored with W3/25⁺ Cells from CY-treated DA Rats with Long-surviving PVG Heart Grafts and/or W3/25⁺ Cells from Normal DA rats

Restorative inocula; number of W3/25 ⁺ cells from cell donors		Rejection time		
CY Rx DA	Normal DA	Animals surviving on day (N) [‡]	Median	p [§]
4 × 10 ⁷	—	18, 29, >100 (6)	>100	NSD
4 × 10 ⁷	5 × 10 ⁶	12, >100 (4)	>100	
—	5 × 10 ⁶	11 (2), 15, 33, 37	15	<0.1
2 × 10 ⁷	5 × 10 ⁶	>100 (3)	>100	<0.1

* DA rats previously treated with CY and bearing long-surviving PVG grafts.

‡ Numbers in parentheses are numbers of individuals.

§ Significance determined by Wilcoxon rank sum test. NSD, not significantly different.

TABLE V
Survival of PVG Heart Grafts in Irradiated Rats Restored with MRC OX8⁺ Cells from Normal or CY-treated DA Rats with Long-Surviving PVG Grafts

Restorative inocula			Rejection time		
MRC OX8 ⁺ (2 × 10 ⁷ cells used)	Normal LN cells (3 × 10 ⁷ cells used)	Normal W3/25 ⁺ cells (5 × 10 ⁶ cells used)	Animals surviving on day (N)*	Median	p [‡]
Normal DA	—	—	>100 (5)	>100	<0.05
CY Rx DA [§]	+	—	12, 13 (2), 14	13	
—	+	—	12, 13 (2), 17 (2)	13	NSD
Normal DA	—	+	7 (3), 9 (2)	7	<0.01
—	—	+	9, 11 (2), 12 (2), 13 (2), 14	12	
CY Rx DA [§]	—	+	9 (2), 11, 14 (2), >100	12.5	NSD

* Numbers in parentheses are numbers of individuals.

‡ Significance determined by Wilcoxon rank sum test. NSD, not significantly different.

§ DA rats previously treated with CY and bearing long-surviving PVG grafts.

cells) did not inhibit the capacity of 3 × 10⁷ normal DA LNC to effect rejection (Table V). MRC OX8⁺ cells from rats with long-surviving grafts were also tested for their capacity to inhibit the restoration of graft rejection by the W3/25⁺ subset of normal LNC. It was found that 2 × 10⁷ MRC OX8⁺ cells from DA rats with long-surviving grafts did not inhibit the ability of 5 × 10⁶ normal W3/25⁺ cells to restore graft rejection (Table V). Thus, these experiments revealed a marked difference between the W3/25⁺ and the MRC OX8⁺ subsets from rats

with long-surviving heart grafts. 2×10^7 W3/25⁺ cells inhibited the restoration of graft rejection by 5×10^6 normal W3/25⁺ cells, but 2×10^7 MRC OX8⁺ cells did not do so. Although 2×10^7 OX8⁺ cells from normal donors apparently increased the alloimmune capacity of normal W3/25⁺ cells (Table V), the same number of OX8⁺ cells from rats with long-surviving grafts did not do so.

Role of B Cells and Sera in Adoptive Transfer of Unresponsiveness. The possible role of B cells in the maintenance of unresponsiveness was examined using the adoptive transfer assay, by transfer of normal LNC mixed with B cells or sera from rats with long-surviving grafts. Neither B cells, nor serum from normal DA rats alone restore rejection (18), nor do they alter the capacity of normal lymphocytes to do so in adoptive transfer assays (Table VI). Thus, B cells and sera from rats with long-surviving grafts were only tested for their capacity to inhibit the allograft reactivity of 3×10^7 normal DA LNC. Neither did so, suggesting that B cell activity does not maintain unresponsiveness in CY-treated rats (Table VI). We established that the assay is suitable for the detection of antibody that can inhibit the rejection response, by demonstrating that serum from an untreated DA rat, which had rejected a PVG graft, specifically inhibited the restorative capacity of normal LNC (Table VI).

Discussion

These experiments showed that rats in which rejection of fully allogeneic cardiac allografts has been prevented by a course of CY treatment develop a state of specific unresponsiveness characterized by an inability of the T helper/inducer cells to initiate rejection. More significantly, they showed that the T helper/inducer cells from these animals have the capacity to inhibit the initiation of the rejection response by normal helper/inducer cells. Neither cytotoxic/suppressor T cells nor antibody appeared to contribute to the maintenance of the state of CY-induced unresponsiveness.

The failure of rats to reject their grafts while receiving CY can be explained by the drug's capacity to inhibit T cell activation. CY apparently inhibits the activation and proliferation of helper/inducer T cells, and thus prevents the production of interleukin 2 and other lymphokines, which are critical for the activation of cytotoxic T cells and B cells (23–29). It does not appear to inhibit the activation of suppressor T cells (27, 29). The failure to effect rejection of the graft and the specific unresponsiveness that develops once CY is stopped cannot

TABLE VI
Survival of PVG Grafts in Irradiated DA Rats Restored with 3×10^7 Normal LNC, and Given Either B Cells or Serum from CY-treated DA Rats with Long-surviving PVG Grafts

Addition to restorative inoculum		Rejection time	
Dose	Source	Animals surviving on day (N)*	Median
4×10^7 B cells	CY Rx DA [‡]	8, 12, 15, 16	13.5
2 ml serum	CY Rx DA [‡]	8 (2), 12 (2), 13 (3), 14, 15	12
2 ml serum	Normal DA	13 (2), 14, 15 (2)	13.5
2 ml serum	DA-rejected PVG [§]	>100 (4)	>100

* Numbers in parentheses are numbers of individuals.

[‡] DA rats previously treated with CY and bearing long-surviving PVG grafts.

[§] From untreated DA rats that had rejected PVG grafts.

be attributed to a direct effect of CY, however. The animals used in our studies had not received CY for at least 75 d, by which time CY is not detectable in any tissues.

Nagao et al. (5), in a detailed study of the development of unresponsiveness in CY-treated heart-grafted rats, showed that the phase of specific unresponsiveness was not manifest until >50 d after cessation of CY treatment, and that the degree of unresponsiveness continued to increase with time. A similar pattern of development of specific unresponsiveness is seen in rats in which graft rejection has been inhibited by passive or active enhancement regimes, prior donor-specific blood transfusion, or treatment with antilymphocyte globulin (1-3, 6, 7). In each of these models, prolonged graft survival is due in part to the development of a state of specific unresponsiveness mediated by a suppressor T cell response (3, 7, 13-16), and in part, to reduced immunogenicity of the implanted graft (15, 30). We suggest that the mechanism of unresponsiveness is similar in all these models, and that in each case, the initial treatment acts by minimizing the helper/inducer T cell response.

The central role of T helper/inducer cells in mediating rejection is highlighted by adoptive transfer experiments in which these cells are the only cells required to restore the capacity of either heavily irradiated or adult thymectomized, irradiated, and bone marrow-reconstituted rodents to reject grafts (17-20). It is still not clear whether the helper/inducer T cells themselves effect rejection, or whether they induce host-derived cytotoxic T cells or B cells to do so (19, 20). Whatever the ultimate mediator is, it is clear that tissue destruction is dependent upon the alloactivation of helper/inducer T cells. It is possible that treatments, which initially inhibit the T helper response to alloantigen, either allow cells of this subclass to transform into antigen-specific suppressor cells, or permit the generation of a new population of W3/25⁺ cells with suppressor potential. The paradoxical finding that cells from rats with specific unresponsiveness associated with long-surviving allografts, which lack the capacity to initiate a rejection response either in the original or adoptive host, have normal reactivity in graft vs. host assays and mixed lymphocyte cultures when reexposed to graft-strain alloantigens remains unexplained (1, 8). It has been suggested (30) that failure of these cells to effect rejection in the original host is due to loss of dendritic cells from the graft. However, a second new graft, not depleted of dendritic cells, given to these animals also fails to induce a rejection response. In our model, the graft in the adoptive recipient is demonstrably sufficiently immunogenic to stimulate small numbers of normal lymphoid cells to effect rejection (18, 20, 22), however, cells from CY-treated animals do not reject it. These findings confirm that a mechanism other than loss of graft immunogenicity is implicated in the long-term survival of organ grafts in CY-treated rats. We suggest that the suppressor cells we have identified are the principal mechanism of unresponsiveness, and that graft adaptation plays only a minor role in long-term survival.

It was clear from our experiments that the T cells that maintain long term unresponsiveness do not belong to the classical cytotoxic/suppressor subpopulation. Suppression mediated by the helper/inducer T cell has also been shown in graft-vs.-host disease (31), tumor allograft models (32), in vitro cytolytic T cell

responses (33, 34), and delayed-type hypersensitivity responses to dinitrofluorobenzene (35).

Others (36, 37) have identified a suppressor cell response in heart-grafted rats treated with CY. The suppressor cells characterized by this group were cyclophosphamide-sensitive, had the capacity to inhibit lymphokine production, and, in contrast to the population identified by us, were apparently of the cytotoxic suppressor subset; partial suppression (but not indefinite graft survival) was transferred to normal Lewis strain adoptive hosts with MRC OX8⁺ cells, but not with W3/25⁺ cells. There is no ready explanation for the difference between these results (36, 37) and ours. However, recent studies (38) on the suppression of antibody responses have suggested that a series of T cells of different phenotypes may be involved in the induction and mediation of suppression. If this is also the case for the suppression of T-mediated responses, the differences between our findings and those previously published (36, 37) may be due to the identification of cells that mediate different parts of the suppressor circuit (38).

Our results strongly suggest that W3/25⁺ cells from grafted CY-treated rats have the capacity to directly suppress normal W3/25⁺ cells. It was clear that neither adoptively transferred normal MRC OX8⁺ cells nor B cells are essential for the induction of suppression in irradiated grafted recipients given W3/25⁺ cells from CY-treated donors. However, although acutely irradiated rats are profoundly lymphocytopenic on the day of grafting, lymphocyte regeneration is obvious by 6–7 d postirradiation (18), and we therefore cannot exclude the possibility that regenerating host cells may be induced by the adoptively transferred W3/25⁺ cells to mediate suppression of the admixed normal W3/25⁺ cells.

The findings we report suggest a central role for the helper/inducer T cell subset in both the mediation of rejection and the maintenance of states of specific unresponsiveness to allografts. Whether both functions are mediated by the same subpopulation, or whether a new population of W3/25⁺ cells replaces the original effector clones remains to be established. Rats with long-surviving grafts are not lymphoid or hemopoietic cell chimaeras (13). However, the functioning graft represents a potential source of alloantigen to prime, induce, and/or maintain the inhibitory response. Whether this is an essential factor in the maintenance of unresponsiveness remains to be established. This form of unresponsiveness induced in allografted adult animals has obvious potential significance for human organ transplantation if means can be devised whereby it can be induced before organ transplantation. Therefore, further investigation of the requirements for its induction are essential.

Summary

DA rats grafted with major histocompatibility complex-incompatible PVG heart grafts and treated with cyclosporine (CY) for 10 d do not reject their grafts, and develop a state of specific unresponsiveness toward PVG allografts. Cells from these animals tested in an adoptive transfer assay were incapable of restoring PVG graft rejection, and capable of specifically inhibiting the capacity of adoptively transferred normal lymph node cells (LNC) to do so. They effected third party Wistar/Furth (W/F) graft rejection, however. Adoptive transfer assays with

purified subpopulations of the lymphocytes that mediated this effect showed that W3/25⁺ T cells of the helper/inducer subclass, when injected alone, failed to restore rejection, and were also able, when injected with normal LNC or the W/25⁺ cells separated from them, to prevent these cells from effecting rejection. MRC OX8⁺ T cells of the cytotoxic/suppressor subclass, B cells, and serum from rats with long-surviving grafts all failed to inhibit the allograft responsiveness of normal LNC, and thus were not identified as mediators of the state of specific unresponsiveness.

These results show that the specific unresponsiveness that develops in rats with long-surviving grafts, and which, in part at least, is responsible for prolonged graft survival, is due to an alteration in the alloreactivity of the helper/inducer subclass of T cells. These cells not only lack the capacity to initiate a rejection response against the alloantigens of the graft, but also have the ability to inhibit the capacity of normal W3/25⁺ cells to do so.

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