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RECIRCULATING, SUPPRESSOR T CELLS IN TRANSPLANTATION TOLERANCE*

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Tolerance to the transplantation antigens which are products of the major histocompatability complex still lacks a universally accepted explanation in cellular terms more than 20 yr since its first experimental induction (1).

A number of phenomena suggest that an active immune response of the suppressor type is present in tolerant animals. A simple deletive mechanism (2, 3) does not explain the failure of large numbers of normal syngeneic cells to reproducibly and regularly break transplantation tolerance (TT)[1] in adoptive transfer experiments (4, 5), the demonstration of specific suppressor cells in animals in which tolerance has recently been terminated (6), or the histological evidence of an ongoing immune response in lymphoid tissue draining a tolerated skin graft (7).

The cells involved in specific suppression of the immune response may be B or T lymphocytes. Antibody is normally detected during B-lymphocyte-mediated suppression (enhancement) differentiating this mechanism from that operating in neonatally tolerant animals. However, it has been suggested that tolerance is in fact the expression of a state of immunological homeostasis in which the forces causing rejection are balanced by protective forces mediated by antibodies (8, 9). Although some workers have demonstrated the co-existence of specific cytotoxic cells and serum borne suppressive factors in neonatally tolerant rats (10) and mice (11), others have been unable to confirm an in vivo role for serum-blocking factors in TT (12, 13).

In 1970 Gershon and Kondo (14) established that under appropriate conditions antigen could stimulate the differentiation of specific suppressor T cells which could induce specific hyporesponsiveness when mixed with normally reactive cells (15).

The consistent failure of various workers to demonstrate suppressive activity in lymphocyte inocula prepared from tolerant animals before the termination of tolerance (13, 16) suggests that if active T-cell suppression is a factor in TT there is a delicate balance between effector and suppressor mechanisms so that no excess suppression is observable unless the balance is upset.

We have examined the possibility that if a sensitive assay system is used, cellular suppressive mechanisms might be demonstrable in transplantation tolerant rats.

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[1] *Abbreviations used in this paper:* BMC, bone marrow cells; DAB/BSA, Dulbecco's phosphate-buffered saline with 0.3% bovine albumin; DA tol PVG, DA strain tolerant of PVG alloantigen; GVH, graft versus host; LNC, lymph node cells; TDL, thoracic duct lymphocytes; TT, transplantation tolerance.

Materials and Methods

Rats. Inbred animals of the DA(Ag-B4), PVG(Ag-B5), Lew(Ag-B1), Aug(Ag-B5), WAG(Ag-B2), and Alb(Ag-B6) strains and their F_1 hybrids were bred in our own laboratories.

Cells. The thoracic duct was cannulated by the method of Gowans and Knight (17). Lymph was collected at 4°C. Lymph node cells (LNC) were obtained by crushing excised nodes in Dulbecco's phosphate-buffered saline (DAB, Oxoid Ltd., London, England) containing 0.3% bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill., DAB/BSA). The suspension was filtered and the cell pellet washed twice. Spleen cells were similarly prepared. Bone marrow cells (BMC) were flushed from the excised femorae and tibiae of donor animals with DAB/BSA containing 100 mg DNase/liter and clumps broken up by repeated pipetting before filtration and washing. All manipulations were at 22°C.

Tolerance Induction. F_1 hybrid BMC were administered to recipients within 24 h of birth in a dose of 5×10^7 cells using the intracardiac injection technique of Grazer (18). Recipients were grafted with skin from female donors of the unshared parental type at 8–12 wk of age and regarded as putatively tolerant if the skin grafts were impeccable 50 days after grafting.

Immunofluorescence. Lymphocyte suspensions were stained for the presence of Ig-bearing cells and cells bearing a T-cell differentiation antigen as previously described (19).

Fc Receptor-Bearing Cells. These were enumerated using the autoradiographic method of Basten et al (20).

Irradiation. To ensure uniformity of tissue distribution of the radiation dose, rats were rotated in a Perspex box at 15 rpm around a vertical axis in the horizontal beam from a ^{60}Co source in a ventilated lead cave under conditions of maximum backscatter. The beam was filtered with shaped lead disks to a dose uniformity across the beam of >96%. The dose was delivered at approximately 40 rads/min.

Graft Versus Host (GVH) Assays. The popliteal lymph node weight assay (21) was used. For screening purposes single dose assays were done.

Cytotoxicity Assays. Cytotoxicity assays were performed in microtiter 2 plates (Falcon Plastics, Oxnard, Calif.) using fresh guinea pig serum as a complement source and aqueous eosin to stain dead cells. Other details were essentially as described by Howard and Scott (22).

Results

Adoptive Restoration of Allograft Reactivity to Irradiated Rats. The details of the adoptive allograft assay have been previously reported (23). Assay animals were irradiated with 750 rads and 24 h later grafted with two mutually Ag-B disparate skin grafts and injected intravenously (i.v.) with the cell populations whose alloimmune competence was being assayed. In all experiments control rats were irradiated and grafted but given no cells.

With the conditions of irradiation described in the Materials and Methods section, although the degree of immunosuppression achieved in individual rats was variable, no animal rejected either of two Ag-B disparate skin grafts before the 12th day (Fig. 1). The injection of 3×10^7 syngeneic thoracic duct lymphocytes (TDL) caused both skin grafts to be rejected by irradiated rats in a normal first set fashion (Fig. 1). The simultaneous application of two mutually incompatible grafts to individual irradiated rats thus provided an assay both for the immunocompetence of restorative inocula and for the specificity of the immunocompetent cells. Proof that the specificity of skin graft rejection was dictated by the restorative inoculum (and not the result of a stimulation of the assay rats own immune system) was the demonstration that parental assay rats restored with inocula of F_1 hybrid lymphocytes were capable of rejecting skin allogeneic both to themselves and the restorative inoculum, but did not reject skin allogeneic to themselves but semisyngeneic with the restorative inoculum. It was in

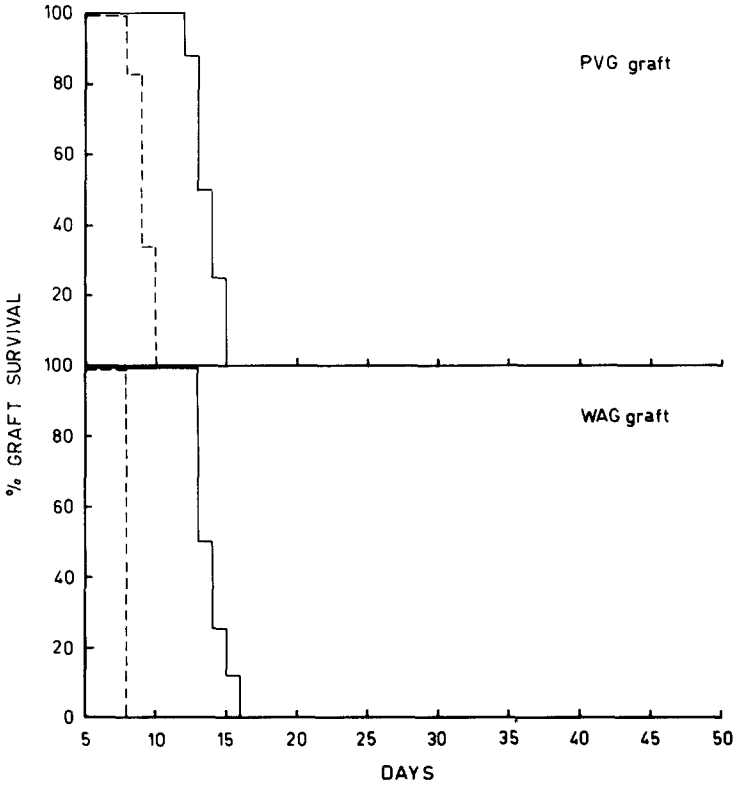

FIG. 1. All irradiated DA rats in this and subsequent experiments were simultaneously grafted with PVG skin on one flank and control third party skin on the other. The percentage of grafted animals bearing intact PVG grafts at each time point is represented in the upper graph and the controls in the lower. Unrestored irradiated controls (—). Rats restored with 3×10^7 normal DA LNC (---).

the course of other experiments designed to confirm the specificity of restoration that evidence suggesting that transplantation tolerance was a positive phenomenon was obtained.

TDL from donors of the DA strain tolerant of PVG alloantigens (DA tol PVG) were assayed in irradiated DA recipients grafted with PVG and WAG skin. Unexpectedly, the PVG skin grafts on these animals survived significantly longer than those on the unrestored irradiated controls which had all rejected both skin grafts by day 16. Six animals in the group retained their PVG grafts for 23, 25(2), 30, 45, and greater than 50 days, respectively. These results implied that cell populations obtained from tolerant donors were capable of specifically abrogating the natural recovery of the allograft response.

Because it was noted that in injected neonatal animals there was some variation in the induction and endurance of the tolerant state, a set of criteria for tolerant donors was established before the adoptive transfer of tolerance was further investigated.

Criteria for Tolerant Donors. Putatively tolerant rats were subjected to a lymph node biopsy, and LNC were assayed for specific absence of GVH activity. All rats tested would have been judged fully tolerant on the criteria of skin graft

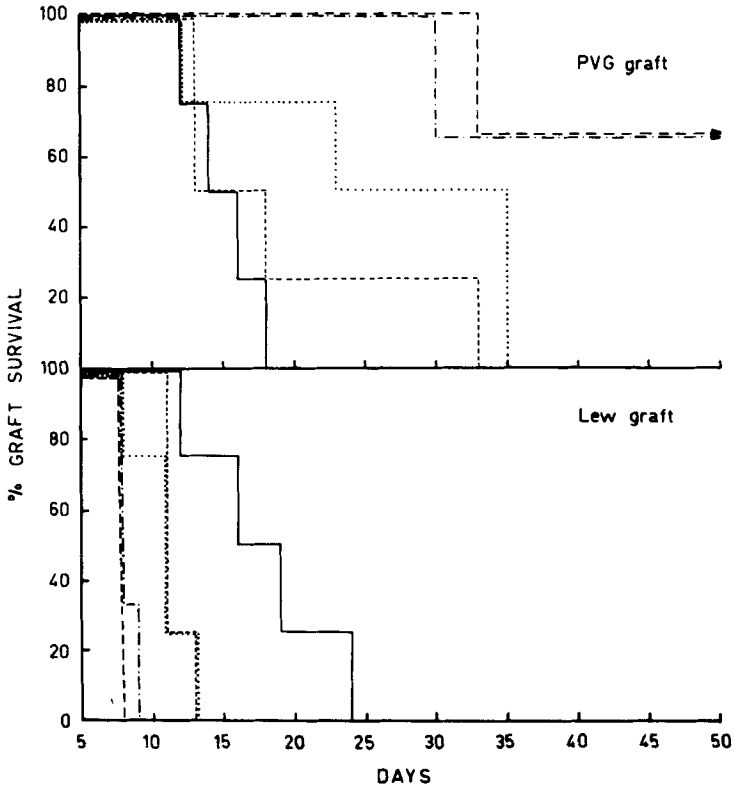

FIG. 2. Restoration of irradiated DA rats with 3×10^7 cells from various tissues of DA tol PVG donors reveals the distribution of suppressor activity. LNC (— — —) and spleen cells (·–·–·–·) show prolonged suppression of PVG graft rejection and prompt restoration of control (Lew) graft rejection. BMC (· · · ·) and thymocytes (– – –) are less efficient at mediating either response. All the tolerant inocula prolonged PVG graft survival and accelerated control Lew rejection compared with the irradiated controls. (—).

survival. Although LNC from the majority were completely unreactive, a minority showed a range of reactivity up to completely normal in one instance. Overall, in these experiments the proportion of putatively tolerant rats showing complete specific abrogation of GVH activity was 84%. Only these rats were used in subsequent experiments.

Distribution of Suppressor Cells in Tolerant Rats. To determine the distribution of the putative suppressor cells the capacity of populations of BMC, thymocytes, spleen cells, and LNC from DA rats tolerant of PVG antigens to specifically suppress the regenerating allograft response was assessed.

The various cell populations were assayed by injecting inocula of 3×10^7 of each into at least six irradiated DA rats bearing PVG and Lew skin grafts. 66% of the recipients of tolerant LNC or spleen cells retained their PVG grafts in perfect condition for more than 100 days. Recipients of tolerant thymocytes and BMC retained their PVG grafts for longer than irradiated unrestored control rats, but none showed permanent acceptance of the grafts. The control Lew grafts on all recipients of cells were rejected before those on unrestored rats (Fig. 2). When these experiments were repeated it was confirmed that permanent

TABLE I
Distribution of Suppressor Cells in Tolerant Donors

Cellular inocula from DA tol PVG donors	PVG graft survival (days) on irradiated DA recipients
Nil	12; 13; 13; 14; 14; 16; 19; 21
5×10^7 BM cells	10; 13; 15; 16; 17; 19; 23
5×10^7 thymocytes	12; 13; 17; 17; 17; 17
1×10^8 thymocytes	12; 13; 17
2×10^8 thymocytes	13; 17
5×10^7 LNC and spleen cells	14; 17; 19; >63; >63; >132; >132
1×10^8 LNC and spleen cells	>84; >132

Where the symbol > is used the recipient either died or was killed with the PVG graft in impeccable condition.

tolerance to alloantigens was not transferred with BMC or thymocytes. Even large inocula of the latter were ineffective although mixtures of LNC and spleen cells from the same donors conferred tolerance on the recipient (Table I).

The finding that among the various lymphoid cell populations LNC, spleen cells, and TDL were very efficient at suppressing the allograft response, and BMC and thymocytes had little effect, suggested that the cells responsible might be members of the recirculating pool.

Identification of Suppressor Cells. Identification of suppressor cells was sought by attempting (*a*) to confirm that the cells were members of the recirculating pool, and (*b*) to determine whether T cells, B cells, or both were necessary for suppression. Experiments previously reported had shown that the lymphoid tissues of rats whose lymphocytes had been virtually eliminated by supralethal doses of radiation were capable of supporting lymphocyte recirculation. Either syngeneic or allogeneic TDL injected i.v. into such rats could be recovered from the thoracic duct uncontaminated by significant numbers of host lymphocytes (24). Cells recovered from the lymph of a recipient after intravenous injection are, by definition, recirculating lymphocytes.

When TDL are injected i.v. into irradiated rats bearing a thoracic duct fistula, the draining lymph remains essentially cell free for 9 h after injection after which time small lymphocytes begin to appear in the lymph in increasing numbers. Effluent cells collected for the 24 h after injection are >99% T cells (19). This method of cell separation was used to prepare T cells from TDL of DA tol PVG donors. Samples of donor TDL were reserved for B-cell strains, GVH assays, and adoptive allograft assays. The remainder were injected i.v. in doses of 1.2×10^9 into four DA recipients which had been irradiated with 900 rads 48 h previously and splenectomized and had thoracic duct cannulae inserted 6 h previously. Collections of lymph were begun at the time of TDL injection (Fig. 3). Cells collected in the first 22 h were pooled and appropriate samples used for Ig stains, GVH assays, and adoptive allograft assays. Cells from the second 22-h drainage were similarly assayed. The results of all three assays of the original inoculum of normal TDL and of the cells collected in the first and the second 22-h collections are presented in Table II. Although Ig^+ cells comprised 44.9% of the TDL from tolerant donors, virtually none of these Ig^+ cells appeared in the lymph of the passage rat within the first 22 h. Assays for Fc receptor-bearing

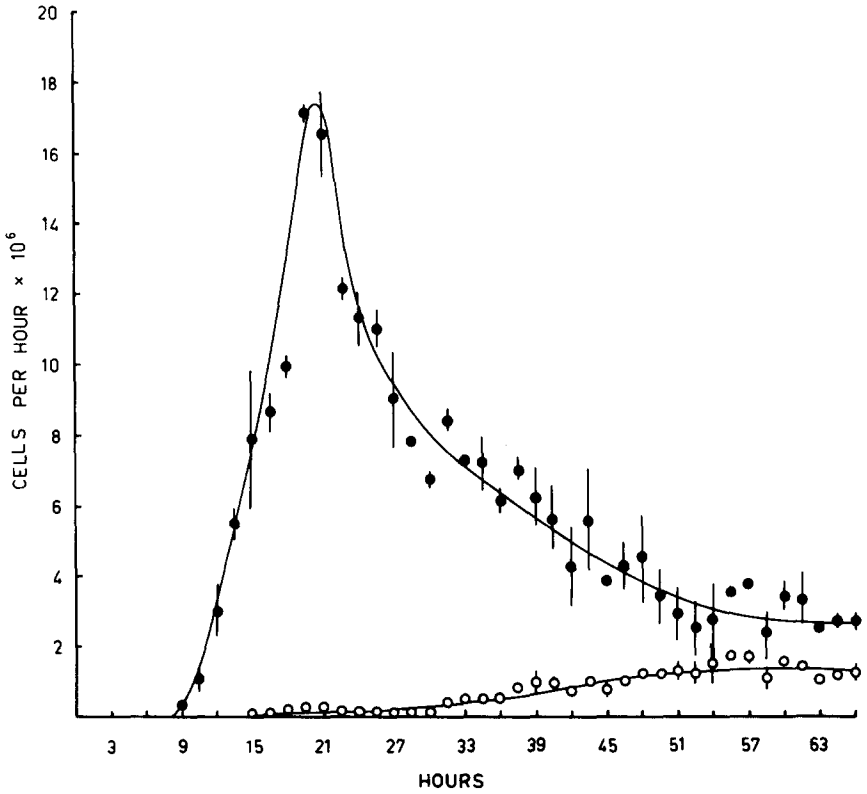

FIG. 3. The output of lymphocytes from the thoracic duct of irradiated (900 rads) DA rats injected i.v. with 1.2×10^9 DA tol PVG TDL at time 0. T-lymphocyte (●) output begins 9 h after injection and peaks at 20–22 h. B-lymphocyte (○) output is negligible until about 30 h after injection. Vertical bars represent the range of values from duplicate animals.

cells showed that the content of these was approximately the same as the content of Ig^+ cells.

The original tolerant TDL, the virtually pure population of T cells obtained after 22-h passage, and the population obtained between 22 and 44 h after passage all behaved in a similar manner in both the GVH and adoptive allograft responses. Each population was incapable of mounting either response against PVG alloantigen, but was normally competent against Lew alloantigen. More importantly, each population was shown to be equally capable of permanently suppressing the recovery of immune competence towards PVG alloantigens.

It was concluded that the suppressive ability of tolerant TDL lies within the rapidly recirculating T-cell population and that B lymphocytes are not necessary for the adoptive transfer of tolerance.

It is theoretically possible that F_1 hybrid, chimeric T cells in the adoptively transferred inocula could have been responsible for the induction of tolerance in the irradiated recipients. This possibility was examined by determining the degree of chimerism in tolerant inocula and the number of F_1 hybrid cells actually needed to induce complete specific tolerance in irradiated rats.

Role of Chimeric Cells in the Adoptive Transfer of Tolerance. The percent-

TABLE II
Transfer of Tolerance by Pure Recirculating T Cells

Source of cell population	Percent B cells	GVH assay			Adoptive allograft assay	
		F_1 hybrid	LN weight ± SE (mg)*		Skin graft	Survival time (days)
			Injected side	Control		
DA tol PVG TDL	44.9	(DA × PVG)	5.0 ± 0.3	4 ± 0.1	PVG	18, 90, >104, >127, >127
		(DA × Lew)	92 ± 8	4.8 ± 0.5	Lew	8, 9, 9, 9, 10
DA tol PVG T cells 0-22 h	0.3‡	(DA × PVG)	5.4 ± 0.2	4 ± 0.3	PVG	17, >104, >104, >104, 117
		(DA × Lew)	112 ± 13	4.7 ± 0.2	Lew	8, 8, 8, 9, 9
DA tol PVG T cells 22-44 h	3.9‡	(DA × PVG)	5.6 ± 0.6	6.2 ± 0.6	PVG	21, 117, >124, >124, 150, >187, >187
		(DA × Lew)	99.2 ± 3.9	8.5 ± 0.8	Lew	8, 8, 8, 8, 8, 8, 8
Irradiated assay rats given no cells					PVG	17, 17, 19, 24, 24
					Lew	13, 15, 19, 19, 19

* Each estimate is the mean of four assay animals injected with 6×10^6 cells.
‡ Non-Ig-bearing cells were also positively identified as T cells as in Materials and Methods. Null cells were not detected.

age cytotoxicity resulting from the incubation of LNC from DA tol PVG rats with Da anti-PVG alloantibody was not above background, indicating that not more than 5% (DA × PVG)F_1 cells were present in the population. On this basis tolerant inocula in the numbers used in the experiments reported here could be assumed to contain a maximum of 2.5×10^6 F_1 hybrid cells.

Groups of irradiated DA rats were grafted with Alb and PVG skin and injected with (DA × PVG)F_1 LNC or purified recirculating T cells in doses varying from 0.5×10^6 to 5×10^7. Control Alb skin grafts on these recipients were rejected in first set time. Animals given 5×10^6 (DA × PVG)F_1 cells or less rejected their PVG grafts with the same tempo as unrestored control rats. One animal given 5×10^7 (DA × PVG)F_1 cells retained the PVG graft in impeccable condition. The others all showed chronic rejection of PVG grafts. It was concluded that the adoptive transfer of tolerance with inocula of 5×10^7 or less cells from tolerant donors was not due to the chimeric cell content of the inocula.

Suppression of Normal Lymphocyte Reactivity by Tolerant Cells. Although experiments had shown specific suppression of the regenerating allograft response in irradiated adoptive recipients, they provided no evidence that suppression was directed at mature peripheral lymphocytes.

A number of attempts were made using different ratios of tolerant and normal cells in adoptive assays to directly demonstrate suppression of the allograft responsiveness of normal lymphocytes. Even in a 10:1 ratio DA tol PVG LNC were unable to suppress normal LNC to the extent of showing permanent survival of PVG grafts on assay rats although they did cause slight delay in the rejection of the grafts.

If, however, challenge of adoptively tolerant recipients with normal cells was delayed for some time, suppression of normal LNC could be demonstrated. A group of adoptive recipients with perfect PVG skin grafts were challenged with either 3×10^7 or 3×10^8 normal DA LNC 3 mo after adoptive transfer of the original tolerance conferring inoculum. Although tolerance was broken in some of these recipients, others clearly demonstrated suppression of the normal cells

TABLE III
Suppression of Normal Challenge LNC by Adoptive Recipients of Tolerant Lymphocytes or Tolerant T Cells

Rat no.	Restorative inoculum from DA tol PVG donor	Challenge inoculum: DA LNC	PVG graft survival after challenge (days)
1	3×10^7 TDL	3×10^7	>30
2	3×10^7 TDL	3×10^8	>30
3	5×10^7 LNC	5×10^7	>55
4	5×10^7 LNC	5×10^7	>55
5	5×10^7 LNC	5×10^7	>55
6	5×10^7 LNC	5×10^7	>55
7	5×10^7 LNC	5×10^7	>55
8	3×10^7 T cells*	3×10^7	12
9	3×10^7 T cells‡	3×10^7	>82
10	3×10^7 T cells‡	3×10^8	>44
11	3×10^7 T cells‡	3×10^8	12

* The 0–22-h collection containing 0.3% Ig + cells.
‡ The 22–44-h collection containing 3.9% Ig + cells.

used for challenge. The experiment was repeated using 5×10^7 cells for challenge, and on this occasion tolerance was not broken in any recipient (Table III).

The Role of the Thymus in Maintaining the Tolerant State

PERSISTENCE OF TOLERANCE IN THYMECTOMIZED RATS. Thymectomy of either neonatally or adoptively tolerant rats did not result in the rejection of tolerated grafts. It is possible, however, that skin grafts are retained on tolerant rats after thymectomy because of a congruent failure to produce both effector and suppressor cells in the absence of a thymus. The suppressor status of thymectomized tolerant rats was therefore examined.

ADOPTIVE TRANSFER OF TOLERANCE FROM THYMECTOMIZED DONORS. The capacity of cells from tolerant donors 4, 5, and 6 mo after thymectomy to transfer tolerance was compared with that of nonthymectomized littermates. Combined data of PVG and control graft survival on recipients given cells from tolerant donors at these times after thymectomy is shown in Fig. 4. Cells from thymectomized donors were less efficient than those from intact donors both at suppressing the response against the tolerated (PVG) alloantigen and mounting a response against the control (Alb) antigen. This latter result showed that thymectomy resulted in a nonspecific loss of effector function in the T-cell pool which had possibly prejudiced the demonstration of specific abrogation of the suppressor status of cellular inocula in adoptive transfer.

INFLUENCE OF THE THYMUS ON THE TERMINATION OF TOLERANCE. The most compelling evidence for potent suppressor function in the T cells of tolerant animals was their ability to confer on adoptive recipients not only prolonged tolerance of the relevant antigen but also resistance to the termination of this tolerance by challenge with normal cells (Table III). In preliminary experiments to test resistance to the termination of tolerance three out of five thymectomized tolerant rats challenged with 5×10^7 normal LNC rejected their grafts within 50 days compared with one out of seven intact tolerant rats similarly challenged.

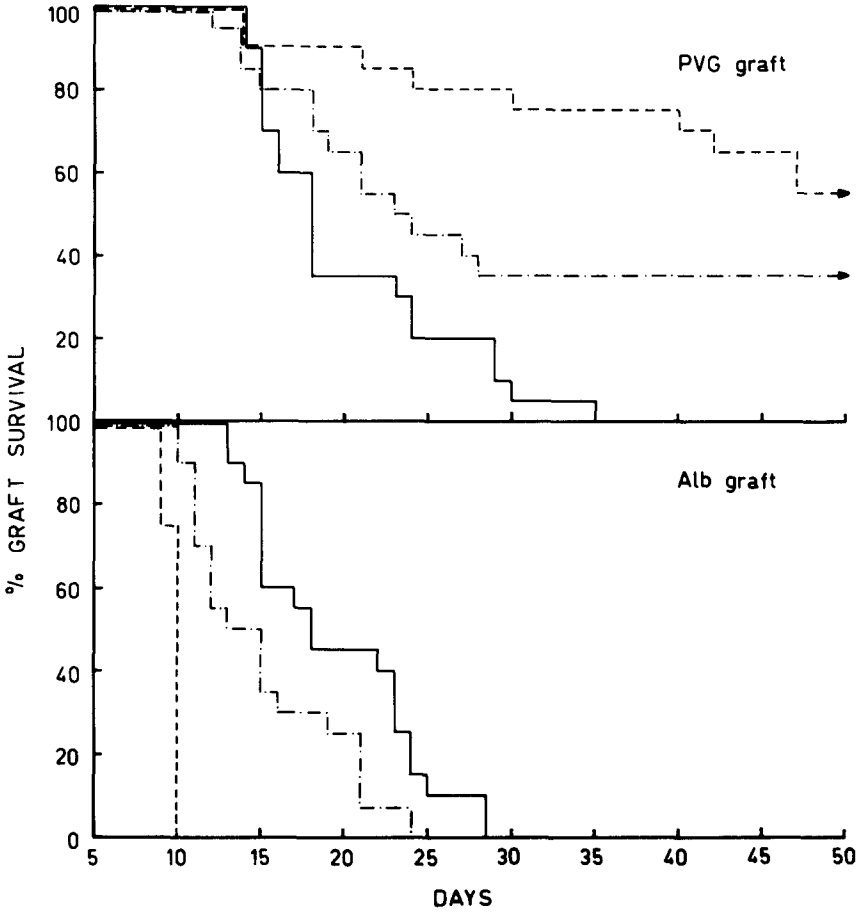

FIG. 4. Restoration of irradiated DA rats with 3×10^7 TDL from thymectomized tolerant donors (·–·–·–·) suppressed PVG graft rejection and accelerated control (Alb) graft rejection less effectively than cells from intact tolerant donors (– – –). Irradiation controls (—).

Discussion

Partial tolerance may be characterized by the presence of serum factors and suppressor T cells not present in true transplantation tolerance (26). It was therefore necessary to establish that the tolerant animals used in the experiments reported in this paper exhibited no in vivo reactivity to the relevant alloantigens. The donors of putatively tolerant cells had already established standard credentials of tolerance by maintaining fully allogeneic skin grafts in impeccable condition for at least 50 days. Tolerance to lymphocyte-determined antigen was established by the failure of large inocula of putatively tolerant cells to react in a quantitative GVH assay. Tolerance to serologically determined antigens was confirmed by the failure of putatively tolerant cells to procure skin graft rejection on adoptive transfer to irradiated hosts.

The adoptive transfer of allograft rejection provides a sensitive assay for the alloimmune capacity of cell populations (23). Irradiation with 750 rads ablates the capacity of a rat to reject Ag-B incompatible skin grafts. Spontaneous

regeneration of this capacity takes at least 12 days. The specificity of the allograft responses restored within this period by intravenous injection of lymphocytes is that of the cell donor and not that of the irradiated recipient. Tolerant lymphocyte populations were capable of specifically and permanently inhibiting the spontaneous regeneration of the allograft response in irradiated rats. This inhibition of recovery could not be explained by clonal deletion in tolerant cell inocula.

A type of specific clonal deletion superficially similar to tolerance can be induced in the recirculating pool of lymphocytes by contact with alloantigens in vivo (24, 29, 30). In irradiated rats inocula of clonally deleted cell populations are incapable of causing the rejection of the relevant skin graft, but they do not suppress the hosts recovery of immunocompetence toward that alloantigen in the manner tolerant cell populations do (24). The prolonged survival of the specific grafts on the adoptive recipients of tolerant cells could not therefore be explained by clonal deletion alone.

Because it has been reported that prolonged graft survival can be induced in immunosuppressed adult animals with allogeneic lymphoid cells (26), the possibility that adoptively transferred tolerance was due to chimeric cells in the tolerant inocula was investigated.

The level of chimerism in tolerant adult rats of the strains used in these experiments has been reported to be very low (31), and cytotoxicity assays confirmed that the lymph nodes from the tolerant donors contained no more than 5% chimeric cells. The fact that small numbers of F_1 cells had no effect on graft survival in irradiated assay rats, and even F_1 cell doses, which were equivalent to 100% chimerism in tolerant cell donors, only procured long-term graft survival in 1 out of 10 recipients makes it extremely unlikely that chimeric cells were responsible for long-term graft survival in animals given inocula of tolerant cells. Furthermore, bone marrow from tolerant donors was ineffective in suppressing irradiated recipients (Table II), indicating that the tissue distribution of suppressor cells is very different from the tissue distribution of F_1 cells capable of inducing long-term transplantation tolerance (32, 33).

The method of T-cell separation used in these experiments (19) depends upon the fact that the modal transit time of T lymphocytes through lymph nodes is considerably shorter than that of B cells (34, 35). Collections of lymph made from heavily irradiated syngeneic rats for the first 22 h after intravenous injection of TDL contain >99% T cells. These pure T-cell populations, and unfractionated TDL from tolerant donors, were both capable of transferring TT. The virtual absence of B cells from the first 22-h collection was established by staining for Ig-bearing and Fc receptor-bearing cells. The cells were positively identified as T cells using a rat anti-T-cell serum (22).

Two features of the T-cell population which mediate suppression in this system require comment. One is that the cells belong to the rapidly recirculating pool of small lymphocytes most of which are long-lived cells (35). This is in contrast to the suppressor T cells which mediate suppression in antibody responses which are short lived and apparently absent from lymph (36). The second is that although these cells may appear strikingly potent (as few as 2% of the recirculating complement will adoptively transfer tolerance) it must be

remembered that the adoptive hosts are irradiated and lymphocytopenic, and thus the initial ratio of suppressor cells to other cells in their tissues is similar to that in the tolerant cell donors. Attempts to produce significant suppression with mixtures of mature peripheral lymphocytes and lymphocytes from tolerant donors were not successful, probably because of the apparent requirement for a fine homeostatic balance between effector and suppressor cells. Indeed the delay in emergence of fully reactive host cells after the termination of tolerance by means of either challenge cells (3) or alloantibody (37, 38) coupled with the observation that this emergence does not occur at all in the absence of the thymus (3) suggest that suppression probably operates at an early point in the immune response, possibly by preventing the generation of antigen-sensitive cells. It seems probable that TT is therefore characterized by clonal deletion maintained by the action of specific suppressor T cells.

When tolerant cells are given to adoptive recipients some time before challenge with normal cells they or their progeny are capable of effective suppression. 10 times the number of cells usually necessary to restore allograft rejection in irradiated assay animals were suppressed in some adoptive recipients of tolerant cells. The events within the adoptive host which lead to this apparent expansion of suppressor activity are unknown but do not necessarily imply proliferation of suppressor cells. The efficacy of suppression may simply lie in the physiological relationship of the suppressor cells with either the tolerated allograft or the lymphoid tissue of the host at the time of entry of challenge cells.

Thymectomy of adult tolerant donors did preferentially depress the adoptive transfer of tolerance, and challenge of these animals suggested that they were more vulnerable to the termination of tolerance than intact tolerant rats. This data is consistent with an extra-thymic lifespan of suppressor cells of similar duration to that of alloantigen-sensitive cells. The partial nature of the effect of thymectomy on both functions suggests that a proportion of each population may also be generated in the periphery from long-lived T cells (39).

McCullagh has recently shown that the thymus in neonatal rats exerts a nonspecific suppressor function for the first 2 days after birth (40), and that prior thymectomy prevents the generation of specific suppressor functions in rats made neonatally tolerant of sheep erythrocytes (41). It seems plausible that persistant exposure to antigen during the phase of generation of T cells from the thymus might well ensure the survival of suppressor cells with specificity for that antigen and the maturation of these cells into the rapidly recirculating, small T lymphocytes described in this paper.

Summary

An adoptive transfer system was used to examine the capacity of cellular inocula from rats fully tolerant of Ag-B antigens to transfer tolerance to irradiated recipients. Permanent tolerance in these irradiated recipients involved specific suppression of the regenerating immune response. Cells obtained from tissues rich in recirculating lymphocytes were the most effective suppressors. Highly purified inocula of T cells from tolerant donors were potent suppressors in irradiated hosts, but were not capable of direct suppression of peripheral antigen-sensitive T cells.

The role of the thymus in maintaining the complement of recirculating suppressor T cells in tolerant animals was examined after adult thymectomy. Thymectomized tolerant rats did not reject their tolerated grafts, and the longevity of the suppression in tolerant rats was confirmed by showing that adoptive transfer of cells from thymectomized tolerant donors was effective in suppressing irradiated recipients up to 180 days after thymectomy. Cellular inocula from these donors appeared to lose their suppressor function marginally faster than they lost effector function (as measured by their capacity to mediate rejection of third party control grafts). Thymectomy made tolerant rats more vulnerable to the termination of tolerance by challenge with normal cells.

Transplantation tolerance is maintained in adult rats by long-lived rapidly recirculating suppressor T cells. The target for the suppressor action of these cells is probably the precursor of alloantigen-sensitive lymphocytes, and the effect of suppression may be deletion or inactivation of the relevant clone of these cells.

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References

1. Billingham, R. E., L. Brent, and P. B. Medawar. 1953. 'Actively required tolerance' of foreign cells. *Nature (Lond).* **172**:603.
2. Burnet, F. M. 1959. Clonal Selection Theory of Acquried Immunity. Cambridge University Press, Cambridge.
3. Elkins, W. L. 1973. Deficit of specific thymus-dependent lymphocytes in transplantation tolerance in the rat. *J. Exp. Med.* **137**:1097.
4. Gowans, J. L., D. D. McGregor, and D. M. Cowen. 1963. The role of small lymphocytes in the rejection of homografts of skin. *In* the Immunologically Competent Cell. *Ciba Found. Study Group.* **16**:20.
5. Billingham, R. E., W. K. Silvers, and D. B. Wilson. 1963. Further studies on adoptive transfer of sensitivity to skin homografts. *J. Exp. Med.* **118**:397.
6. Elkins, W. L. 1972. Cellular control of lymphocytes initiating graft vs. host reactions. *Cell Immunol.* **4**:192.
7. Awaya, K., and P. McCullagh. 1973. The histological changes in immunologically tolerant lymphoid tissue. *Aust. J. Exp. Biol. Med. Sci.* **51**:737.
8. Voisin, G. A. 1971. Immunity and tolerance: a unified concept. *Cell Immunol.* **2**:670.
9. Voisin, G. A., R. G. Kinsky, and H. T. Duc. 1972. Immune status of mice tolerant of living cells. II. Continuous presence and nature of facilitation-enhancing antibodies in tolerant animals. *J. Exp. Med.* **135**:1185.
10. Wright, P. W., I. D. Bernstein, B. Hamilton, and K. E. Hellstrom. 1975. In vitro reactivity in allograft tolerance. Persistance of cell-mediated cytotoxicity and serum blocking activity in highly tolerant rats. *Transplantation (Baltimore).* **19**:437.
11. Hellstrom, I., K. E. Hellstrom, and A. C. Allison. 1971. Neonatally induced allograft tolerance may be mediated by serum-borne factors. *Nature (Lond.).* **230**:49.
12. Brent, L., C. Brooks, N. Lubling, and A. V. Thomas. 1972. Attempts to demonstrate an in vivo role for serum blocking factors in tolerant mice. *Transplantation (Baltimore).* **14**:382.
13. Brooks, C. G. 1975. Neonatally induced transplantation tolerance: in vitro evidence supporting a clonal inactivation mechanism. *Eur. J. Immunol.* **5**:741.

14. Gershon, R. K., and K. Konda. 1970. Cell interactions in the production of tolerance: the role of thymus lymphocytes. *Immunology*. **18**:723.
15. Gershon, R. K., P. Cohen, R. Hencin, and S. A. Liebhaber. 1972. Suppressor T cells. *J. Immunol.* **108**:586.
16. Atkins, R. C., and W. L. Ford. 1972. The effect of lymphocytes and serum from tolerant rats on the graft-versus-host activity of normal lymphocytes. *Transplantation (Baltimore)*. **13**:442.
17. Gowans, J. L., and E. J. Knight. 1964. The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. B. Biol. Sci.* **159**:257.
18. Grazer, R. M. 1958. Technic for intravascular injection and bleeding of newborn rats and mice. *Proc. Soc. Exp. Biol. Med.* **99**:407.
19. Dorsch, S., and B. Roser. 1975. T cells mediate transplantation tolerance. *Nature (Lond.)*. **258**:233.
20. Basten, A., J. F. A. P. Miller, J. Sprent, and J. Pye. 1972. A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. *J. Exp. Med.* **135**:610.
21. Ford, W. L., W. Burr, and M. Simonsen. 1970. A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. *Transplantation (Baltimore)*. **10**:258.
22. Howard, J. C., and D. W. Scott. 1974. The identification of sera distinguishing marrow-derived and thymus-derived lymphocytes in the rat thoracic duct. *Immunology*. **27**:903.
23. Dorsch, S., and B. Roser. 1974. The adoptive transfer of first set allograft responses by recirculating small lymphocytes in the rat. *Aust. J. Exp. Biol. Med. Sci.* **52**:33.
24. Dorsch, S., and B. Roser. 1974. The clonal nature of alloantigen-sensitive small lymphocytes in the recirculating pool of normal rats. *Aust. J. Exp. Biol. Med. Sci.* **52**:45.
25. Sprent, J., H. von Boehmer, and M. Nabholz. 1975. Association of immunity and tolerance to host *H-2* determinants in irradiated F_1 hybrid mice reconstituted with bone marrow cells from one parental strain. *J. Exp. Med.* **142**:321.
26. Brent, L., C. G. Brooks, P. B. Medawar, and E. Simpson. 1976. Transplantation tolerance. *Br. Med. Bull.* **32**:101.
27. Elkins, W. L., I. Hellstrom, and K. E. Hellstrom. 1974. Transplantation tolerance and enhancement. *Transplantation (Baltimore)*. **18**:38.
28. Brooks, C. G. 1976. Induction of neonatal transplantation tolerance. Do MLC antigens provide the major barrier? *Transplantation (Baltimore)*. **21**:347.
29. Ford, W. L., and R. C. Atkins. 1971. Specific unresponsiveness of recirculating lymphocytes after exposure to histocompatability antigen in F_1 hybrid rats. *Nat. New Biol.* **234**:178.
30. Sprent, J., J. F. A. P. Miller, and G. F. Mitchell. 1971. Antigen induced selective recruitment of circulating lymphocytes. *Cell Immunol.* **2**:171.
31. McCullagh, P. 1975. Suppressor cells in homograft tolerant rats. *Aust. J. Exp. Biol. Med. Sci.* **53**:431.
32. Barker, C. F., D. M. Lubaroff, and W. K. Silvers. 1971. Lymph node cells: their differential capacity to induce tolerance of heart and skin homografts in rats. *Science (Wash. D. C.)*. **172**:1051.
33. Silvers, W. K., and R. E. Billingham. 1969. Influence of the Ag-B locus on reactivity to skin homografts and tolerance responsiveness in rats. *Transplantation (Baltimore)*. **8**:167.
34. Ford, W. L., and S. J. Simmonds. 1972. The tempo of lymphocyte recirculation from blood to lymph in the rat. *Cell Tissue Kinet.* **5**:175.
35. Howard, J. C. 1972. The life-span and recirculation of marrow derived small lymphocytes from the rat thoracic duct. *J. Exp. Med.* **135**:185.

36. Ha, T-Y., and B. H. Waksman. 1973. Role of the thymus in tolerance. X. 'Suppressor' activity of antigen-stimulated rat thymocytes transferred to normal recipients. *J. Immunol.* **110**:1290.
37. Lubaroff, D. M., and W. K. Silvers. 1970. The abolition of tolerance of skin homografts in rats with isoantiserum. *J. Immunol.* **104**:1236.
38. Harris, T. N., S. Harris, and M. B. Farber. 1973. Effect of antigraft strain antibodies on skin allografts in immunologically tolerant mice. *Transplantation (Baltimore).* **15**:383.
39. Taylor, R. B., and A. Basten. 1976. Suppressor cells in humoral immunity and tolerance. *Br. Med. Bull.* **32**:152.
40. McCullagh, P. 1975. Role of the thymus in suppression of immune responses in newborn rats. *Aust. J. Exp. Biol. Med. Sci.* **53**:413.
41. McCullagh, P. 1976. Modification of the tolerant state by neonatal thymectomy. *Immunology.* **31**:39.