THE CELLULAR BASIS OF ALLOGRAFT REJECTION IN VIVO

I. The Cellular Requirements for First-Set Rejection of Heart Grafts*

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Graft rejection does not occur in vivo in the absence of T cells (1–4). It is not known however whether T cells alone can mediate graft rejection. Indeed the invariable association of B-cell responses, including alloantibody synthesis, with graft rejection in normal animals suggests that the requirements for rejection may be complex and at least partly B-cell dependent (4–6). However, the association of a particular immune response with graft rejection does not necessarily imply that it is a requirement for rejection (5). The cellular requirements for graft rejection in vivo can be directly established by adapting the adoptive transfer technique (7) and testing known cell populations singly or in combination for their ability to cause rejection in irradiated animals bearing allografts (8–10).

Large doses of irradiation are required to ensure consistent ablation of first-set allograft responses (8). As the rat is very sensitive to the rapidly-fatal gastrointestinal syndrome after conventional irradiation techniques (11) successful adaptation of the adoptive transfer system to study of the response to antigens of the major histocompatibility complex (MHC)[1] depends on careful attention to the parameters of irradiation to ensure a uniform whole-body dose close to the LD10/30 days. Under such optimal conditions the irradiated rats provide an immunologically-inert milieu in which the host cells make no detectable contribution to the specificity of the immune response and the immune potential of adoptively transferred cells can be accurately determined (7, 8). Using microsurgical anastomosis of adult heart grafts in such animals we have previously shown that the potency of adoptively transferred inocula of lymphocytes can be measured with precision (10). The cellular requirements for first-set graft rejection in the rat have now been studied with the same assay system.

Materials and Methods

Animals. Inbred rats of the strains PVG-H-1^c (Ag-B5), LEW H-1^e (Ag-B1), DA H-1^a (Ag-B4), BN H-1^n (Ag-B3), and AUG H-1^c were bred in our own animal house. Nomenclature of rat strains is from Rat Newsletter, no. 3, 1978; Medical Research Council Laboratory Animals Center.

Irradiation. Animals lying horizontally in a Perspex box were rotated at 16 rpm around the vertical axis in a horizontal beam from a ^{60}Co source at a distance of 25 cm. The beam was filtered with shaped lead discs to an isodose uniformity of $>96\%$ and was directed into a lead cave where irradiation occurred under conditions of maximum backscatter, at a dose rate of 50

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[1] *Abbreviations used in this paper:* DAB/BSA, Dulbecco's phosphate-buffered saline containing 0.3% wt/vol bovine serum albumin; HU, hydroxyurea; Ig+, lymphocytes bearing surface immunoglobulin detectable with fluoresceinated rabbit antibodies to rat immunoglobulins; LNC, lymph node cells; MHC, major histocompatibility complex; TDL, thoracic duct lymphocytes; VBL, vinblastine sulphate.

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rads/min. The irradiation time was calculated by a program which corrected for isotope decay and was timed electronically to a reproducibility of >99.9%.

Heart Grafting. Hearts were removed from adult donors immediately after an intravenous injection of 50 U of preservative-free heparin. Donors had been irradiated with 750 rads within the previous 6 h. Hearts were anastomosed to the abdominal great vessels by a modification of the technique of Ono and Lindsey (12). Irradiated recipients of heart grafts were given 750 rads, the optimal dose for ablation of the allograft response (8), within 6 h before surgery. The end point of graft rejection was loss of palpable heart beat confirmed by loss of electrocardiogram activity.

Statistics. Because the rejection times of grafts in experimental groups of animals fall within a narrow range of values it is appropriate to express results in terms of mean survival values ± a standard error and to estimate the significant differences between groups using the Student's *t* test.

Cell Preparations. Thoracic duct lymphocytes (TDL) were obtained from an indwelling cannula by the method of Gowans and Knight (13). Lymph was collected at 4°C. Cells were washed twice by centrifugation in Dulbecco's phosphate-buffered saline with calcium and magnesium (DAB, Oxoid) containing 0.3% bovine serum albumin (Armour Pharmaceutical Co. Ltd., Hampden Park, East Bourne Sussex, U.K.) (DAB/BSA). Single cell suspensions were prepared from pooled cervical and mesenteric lymph nodes or spleen by crushing between the flatground bottom of a 50-ml beaker and the floor of a Petri dish containing DAB/BSA. Thymocytes were obtained by the same method. Thymus donors were injected intraperitoneally with 1 ml of a 1:100 dilution of colloidal carbon (Pelikan 518 Gunther Wagner) 24 h previously to stain the parathymic lymph nodes which were carefully removed before crushing. Bone marrow cells were flushed from the tibiae and femora using DAB/BSA and clumps broken up by repeated pipetting. All cell suspensions were filtered through four layers of sterile surgical gauze before washing twice in DAB/BSA. Dead cells were removed where necessary by flotation on isotonic 35% BSA solution pH 7.4 (14).

In Vitro Cell Separation. Lymphocytes negative for membrane-bound immunoglobulin (Ig-) were obtained from cell preparations by selective adherence of immunoglobulin-bearing (Ig+) B cells to affinity-purified, sheep anti-rat IgG, immobilized on solid supports. Supports were either columns of coarse G50-Sephadex activated with periodate by a modification of the method of Wilson and Nakane (15) or tissue culture grade Petri dishes (BioQuest, BBL & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.) coated by the method of Mage et al. (16). In both techniques the yield of T cells was 69–90% and contamination with Ig+ cells was less than 5%.

Immunofluorescence. Cell-membrane immunofluorescent stains were done at 22°C in DAB/BSA containing 0.02 M sodium azide, on cell suspensions at 10^8/ml which were >90% viable by nigrosine exclusion. Preparations were stained for Ig+ cells with fluorescent rabbit anti-rat immunoglobulin (Burroughs Wellcome). Cells were stained for 30 min, washed once in DAB/BSA, then resuspended in 3 ml DAB/BSA - azide and layered on 4 ml 6% BSA in DAB also containing 0.02 M azide and pelleted by centrifugation at 350 *g* for 10 min. Careful aspiration of the supernate removed all non cell-bound reagent. The pellet was resuspended in one to two drops of 6% BSA-azide, smeared, dried, and fixed for 10 min in absolute reagent-grade ethanol before drying and mounting in glycerol buffered to pH 9.8. Slides were examined with a Leitz microscope with Ploempak incident-light fluorescence and combined transmitted-light phase contrast optics. Where cell preparations contained erythrocytes these were removed after fixation by immersing the slides for 1–2 min in distilled water containing 13 g/liter cetrimide and washing thoroughly in distilled water. This treatment completely lysed erythrocytes and did not remove either fluorescent stain or nucleated cells. T-cell markers were demonstrated by two stage techniques using fluorescent anti-Ig reagents at the second-stage. After first stage staining the cells were washed once in DAB/BSA before layering on 6% BSA and centrifuging to ensure complete removal of non cell-bound antibody before second-stage staining with anti-immunoglobulin. First stage reagents were (*a*) AUG anti-PVG alloantisera prepared by the method of Howard and Scott (17) to detect the Pta antigens, A2 and B (not 1), which are present only on peripheral T cells in the PVG rat[2] and W3/13 antibody recently described by

[2] G. W. Butcher and J. C. Howard. Rat Newsletter, no. 1. M. Festing, editor. MRC Laboratory Animal Center.

Williams et al. (18) produced by mouse-mouse hybrid plasma cell clones in vitro. W3/13 binds to both thymocytes and peripheral T cells in the rat, (*b*) a rabbit anti-Thy-1.1 antiserum, which had been extensively absorbed to render it specific for Thy-1.1. This reagent binds strongly to rat thymocytes (19) and binding to TDL can be detected using a polyvalent anti-Ig second stage reagent (20). The second stage reagent for anti-Pta antibody and specificity W3/13 was fluorescent rabbit anti-rat Ig and for Thy-1.1 antibody was sheep-anti-rabbit Ig.

Plasma cells in histological sections were stained directly with the fluorescent rabbit anti-rat Ig reagent. Tissues were fixed for 1 h at 22°C in 70% ethanol, glacial acetic acid, and 40% formaldehyde solution in a volumetric ratio of 20:1:1, embedded in paraffin wax, and sectioned at 5–7 μm.

Recirculating T lymphocytes were prepared by injecting 10^9 TDL, lymph node cells (LNC), or spleen cells intravenously into irradiated (900 rads) syngeneic rats bearing a thoracic duct cannula. The cells which emerged from the cannula within 24 h of injection were >95% T cells as judged by absence of surface Ig, the presence of Pta antigen, and positive staining with specificity W3/13.

Mitotic Inhibition. Vinblastine (VBL) was given by intravenous injection at a dose of 0.3 mg/rat daily for two doses. Hydroxyurea (HU) was given twice daily in doses of 250 mg/kg for 4 days.

Cytotoxic alloantibodies were titrated with a modification of the Eosin exclusion method of Howard and Scott (17).

Results

First Set Graft Rejection in Normal Recipients and the Effect of Irradiation. Rats of all the strains tested rejected hearts which differed at the major histocompatability complex in 6–8 days. Irradiation of recipients ablated the capacity to reject H-1 incompatible grafts. Irradiated PVG recipients did not reject H-1 incompatible heart grafts for at least 40 days. Although there was an inevitable mortality of about 15% in grafted recipients, no insurmountable surgical problems were encountered in grafting irradiated rats. Irradiated PVG rats grafted with hearts from DA donors were used to assay the capacity of cellular inocula prepared from PVG donors to restore graft rejection.

Restoration of Graft Rejection with Lymphoid Cells. The ability of cellular inocula, prepared from various lymphoid organs, to restore graft rejection was assayed. Initially, varying numbers of LNC were injected intravenously into irradiated PVG recipients grafted with DA hearts. It was found that the dose of lymphocytes used to restore irradiated recipients correlated with the duration of graft survival. 5×10^8 LNC restored graft rejection to a tempo approximating that in intact rats. Fewer cells took longer to restore graft rejection. Increasing the dose beyond 5×10^8 cells did not accelerate rejection beyond 8–10 days which therefore represents a maximum restorative effect (Table I). This suggests that with 5×10^8 cells the assay rat is saturated with effector cell precursors and at this dose and above the time taken to reject the graft reflects the duration of the physiological and immunological reactions required before tissue destruction is completed in irradiated recipients. This time is invariably 1–2 days longer than first-set rejection in normal animals. This probably reflects the time required for adoptively transferred cells to regain their normal physiological relationship with the host lymphoid tissue. The demonstrable quantitative relationship between the number of cells given and the duration of graft survival meant that the adoptive transfer system could be used to measure the relative number of effector cell precursors in different restorative inocula. Therefore using cell doses of 5×10^8 and less the restorative potency of cells from various tissues was titrated (Table I).

LNC and TDL are both potent in restoring rejection. Thymocytes and bone marrow are not. Unexpectedly the spleen was also deficient in cells competent to

TABLE I

Heart Graft Rejection in Irradiated PVG Rats Restored with Cells from Various Lymphoid Tissues*

Source	Restorative inoculum				
	10^9	5×10^8	2×10^8	5×10^7	2×10^7
LNC	9.6 ± 0.7 (5)‡	9.6 ± 0.8 (6)	14.3 ± 0.6 (6)	20.5 ± 1 (6)	31.4 ± 1.6 (5)
TDL	ND	8.4 ± 0.2 (5)	11 ± 2.3 (5)	ND	21.6 ± 3.3 (5)
Spleen	ND	21 ± 1.1 (4)	28 ± 0.8 (7)	ND	36.2 ± 1.5 (5)
Thymus	45 ± 3.2 (5)	ND	54 ± 2.6 (4)	ND	ND
Bone marrow	ND	ND	69.6 ± 2.5 (4)	ND	ND

‡ Graft survival is expressed as the mean survival time (MST) of the group ± SEM (number in group).
* MST of grafts in irradiated PVG recipients not reconstituted with cells was 59 ± 5.3 (15).

mediate graft rejection. To achieve equivalent rejection tempo required 10 times more spleen cells than LNC. TDL appeared slightly more potent than LNC. As evidence presented later in this paper indicates that the spleen, like the thymus, and bone marrow contains relatively few recirculating lymphocytes, the distribution of cells able to cause graft rejection indicated by these assays is compatible with the thesis that effector cell precursors belong to the recirculating pool. It gives no information as to which of the subpopulations within this pool they represent.

Roles of T and B Cells in Graft Rejection. Using the in vitro techniques described in Materials and Methods inocula of purified Ig− lymphocytes were prepared from TDL and LNC populations and assayed for their ability to restore graft rejection. The effective absence of B cells in the purified inocula was confirmed by measuring the titer of specific alloantibody synthesis restored to adoptive recipients. Ig− cells, given in doses equivalent to the T-cell content of unfractionated cell populations restored graft rejection with the same tempo as the unfractionated inocula. 2×10^7 unfractionated LNC caused rejection in 25.3 ± 1.9 days. The Ig− cell equivalent (on the basis that 65% of LNC are Ig−) caused rejection in 27.8 ± 1.2 days ($0.4 > P > 0.3$). While no antibody was detected in the recipients of purified Ig− cells, recipients of unfractionated cellular inocula showed titers of alloantibody only 4–8-fold lower than those of normal PVG rats grafted with DA skin (Fig. 1). The presence or absence of antibody in the serum did not affect graft rejection. Histological sections of rejected heart grafts from normal animals or those given Ig− cells were scanned and the number of plasma cells/section counted. The former showed a profuse cellular infiltrate containing 50–200 plasma cells per cross-section of myocardium at the midpoint of the long axis of the ventricles. The virtual absence of plasma cells in similar sections of rejected tissue from Ig− cell reconstituted rats, made it unlikely that antibody produced locally was effecting rejection in these recipients.

The ability of purified Ig− cells to secure graft rejection and the high concentration of effector cell precursors in tissues rich in recirculating lymphocytes suggested that a recirculating T cell might be the essential mediator of first-set graft rejection.

Role of Recirculating Cells in Graft Rejection. Rapidly recirculating T cells were separated from normal populations of LNC, spleen cells, and TDL and their potency in graft rejection examined as follows: 10^9 cells from each population was injected into heavily irradiated syngeneic recipients bearing a thoracic duct fistula and timed collections of the cells emerging from the fistula were begun. B-cell stains were done on samples from each 90 min collection and the rate of emergence of T and B cells calculated (Fig. 2). Spleen cell suspensions yielded significantly fewer recirculating T

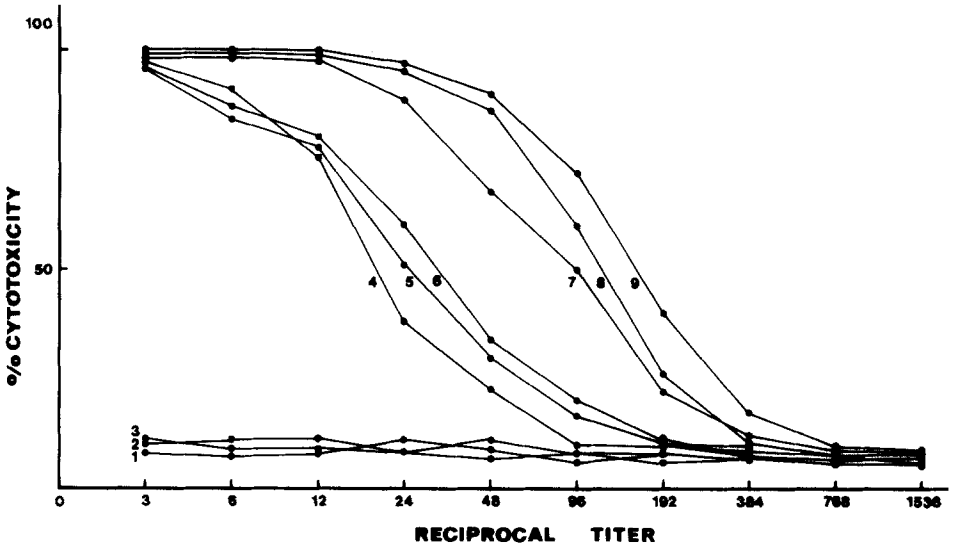

FIG. 1. Serum cytotoxic antibody titrations of individual HO rats which have rejected DA hearts. Serum was taken on the day of rejection. 1, 2, 3. Irradiated rats restored with 7.5×10^7 recirculating T cells (mean survival time [MST] 11 days). 4, 5, 6. Irradiated rats restored with 1.5×10^8 unfractionated TDL (MST 11 days). 7, 8, 9. Nonirradiated rats (MST 8 days).

cells than LNC or TDL. The peak output of cells from the thoracic duct of animals injected with spleen cells was 2.3×10^6/h and the total T-cell output per recipient over the 48-h of collection 7.9×10^7. This is in contrast to a peak output of 1.71×10^7 and a total recovery of 2.87×10^8 T cells from animals injected with LNC and a peak output of 1.33×10^7/h and total recovery of 3.23×10^8 T cells from animals injected with TDL (Fig. 2). The rapidly recirculating cells from each source were positively identified with T-cell-specific stains (Table II). In all three groups >95% of cells collected in the first 22 h drainage were T cells. Both unfractionated TDL and the rapidly recirculating fraction of cells showed uniform staining of the T cells with anti-Pta and specificity W3/13 as expected. On unfractionated TDL the second-stage reagent (fluorescent rabbit anti-rat Ig) also stained B cells resulting in staining of all cells in the preparation. With the anti-Thy-1.1 reagent a subpopulation which did not bear Thy-1.1 was highly enriched in the rapidly recirculating T-cell pool (85%) but comprised only a minority of the cells in TDL (14%). Because the second stage reagent (fluorescent sheep anti-rabbit Ig) did not cross-react with surface Ig on rat B cells it was possible to establish that a proportion of B cells in TDL must also bear Thy-1.1.

When the restorative capacity of the three populations of recirculating T cells was compared they were found to be equipotent. 10^7 recirculating T cells from spleen caused heart graft rejection with the same tempo as 10^7 recirculating T cells from TDL or LNC. This is in contrast to the relative potency of the unseparated cell population from each of these sources (Table III). The first 22 h collection of recirculating cells from the intermediate hosts injected with LNC contained 1.56×10^8 cells which was about seven times greater than the number in recirculating cells obtained from spleen (2.3×10^7). The restorative potency of unfractionated LNC

was about 10 times the potency of unfractionated spleen cells. This is sufficiently good agreement to ascribe rejection to the recirculating T-cell content of these inocula.

It was concluded that: (*a*) spleen cell suspensions contain a significantly smaller proportion of recirculating cells than either TDL or LNC and the poor restorative capacity of spleen cell populations is a reflection of this. (*b*) The recirculating T cells obtained from spleen LNC and TDL are equipotent in graft rejection. (*c*) The cells in spleen, lymph nodes, and thoracic duct lymph which cause heart graft rejection are recirculating T cells.

Smears of the rapidly recirculating cells showed that more than 99% of them were typical small lymphocytes. Although these are known to be predominantly long-lived, experiments were done to determine whether the effector cell precursors were derived from this predominant pool or from a minority pool of short-lived cells.

Life Span of Effector Cell Precursors

ROLE OF THE THYMUS. The failure of neonatally thymectomized or congenitally athymic mice to reject grafts indicates that the precursor cell, like all T cells, is ultimately dependent on the thymus for its production. That adult thymectomy has little effect on the capacity of intact animals to reject allografts does not necessarily mean that the adult thymus does not play a role in maintaining the size of the pool of effector cell precursors throughout life. Loss of a continuing small output of long-lived cells from the thymus after adult thymectomy would not be evident for many months because of the redundancy of effector cell precursors in normal animals (Table I).

The potency of inocula of cells prepared from the lymph nodes and thoracic duct lymph of donors thymectomized 3 mo previously was examined. These cells were, if anything, more potent in graft rejection than cells from normal donors (Table IV). It is apparent that the effector cell precursor is independent of thymic influence after the neonatal period and that cells recently derived from the thymus and seeded into the recirculating pool are inactive in graft rejection and in fact dilute the pool of effector cell precursors. This result gives no information as to whether effector cell precursors are individually long-lived cells or are derived from dividing T cells in the periphery which are independent of thymic influence.

Longevity of Effector Cell Precursors. When given according to the regime outlined in Materials and Methods, VBL has been shown to ablate immune responses mediated by short-lived lymphocytes, while leaving unaffected those responses mediated by long lived cells (21). The dose of HU required to abolish DNA synthesis in the donors was established by titrating the degree of depression of tritiated thymidine uptake of bone marrow cells against increasing drug dosage. The regime finally adopted resulted in >95% depression of thymidine uptake and a reduction of 90% in the nucleated cells present in the marrow.

LNC from treated animals showed no loss of GVH activity when compared with LNC of normal rats in the popliteal lymph node weight assay of Ford et al. (22). The effect of these inhibitors and the capacity of lymphocytes from naive donors to restore heart graft rejection is seen in Table IV. Restorative inocula prepared from animals treated with either agent showed no decrease in their potency to restore heart graft rejection indicating that effector cell precursors were not recently derived from mitotically active progenitor cells. Taken with the ineffectiveness of adult thymectomy

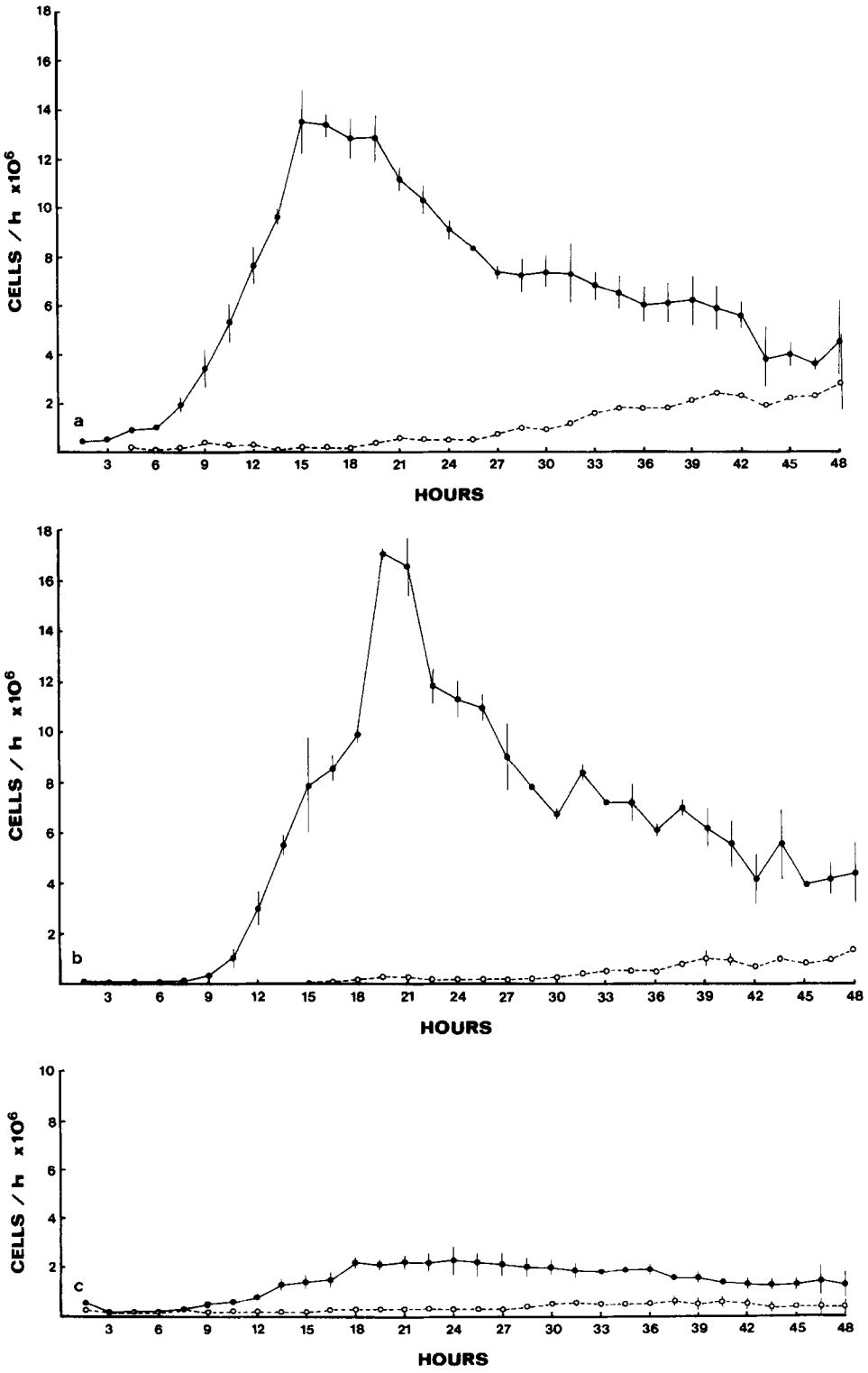

FIG. 2

TABLE II

*T-Cell Markers on TDL and on Recirculating T Cells**

Membrane antigen	First stage reagent	Second stage‡ reagent (fluorescent)	Fluorescent cells	
			TDL	Recirculating T cells
			(%)	
Ig	—	Rab α-rat Ig	46	1.5
W3/13 epitope	Specificity W3/13§	"	98	99
Pta A2 B (not 1)	AUG α-HO serum ‖	"	100	100
Control	—	Sh α-Rab Ig	1	0
Thy-1.1	Rab α-Thy-1.1¶	"	86	15

* Recirculating cells recovered from the lymph within 24 h of intravenous injection into irradiated cannulated hosts.
‡ Concentration, 0.3 g/liter.
§ 1.0 g/liter.
‖ 1:4.
¶ Undiluted.

TABLE III

Comparison of the Potency of Recirculating T Cells from Various Lymphoid Tissues

Unfractionated			Recirculating T cells		
Source	Inoculum	Rejection time	Source	Inoculum	Rejection time
TDL	1.5×10^8	11 ± 0.3 (5)	TDL	7.5×10^7	10.9 ± 0.4 (9)
"	2×10^7	21.6 ± 3.3 (5)	"	10^7	26.4 ± 2.5 (5)*
LNC	2×10^7	31.4 ± 1.6	LNC	10^7	25.5 ± 2.3 (5)‡
Spleen	2×10^7	36.2 ± 1.5	Spleen	10^7	24.8 ± 2.1 (5)§

*, ‡, § These groups are not significantly different (1,2 = *, ‡; $0.4 > P > 0.3$), (2,3 = ‡, §; $0.4 > P > 0.3$), (1,3 = *, §; $0.4 > P > 0.3$).

TABLE IV

Effect of Thymectomy or Anti-Mitotic Agents on Potency of Restorative Inocula of LNC

Restorative inoculum	Treatment	Rejection time
1.5×10^8	—	14.3 ± 0.6 (6)
1.5×10^8	ATX*	10 ± 0.4 (4)
2×10^7	—	24.5 ± 3.3 (4)
2×10^7	ATX*	20 ± 0.7 (5)
2×10^7	VBL	18 ± 1.1 (4)
2×10^7	HU	18.3 ± 1.5 (4)

* Donors were thymectomized at 12 wk of age 3 mo before donation of cells.

FIG. 2. The in vivo separation of recirculating T cells from (a) TDL (b) LNC (c) spleen cells. 10^9 cells from the population to be separated were injected intravenously into irradiated (900 rads) syngeneic intermediate hosts bearing a thoracic duct fistula and timed collections of lymph were begun. Samples from each 1.5 h collection were stained for the presence of Ig+ cells. Each graph plots the mean output per h of recirculating T cells (●) and B cells (○) from at least four intermediate hosts.

in reducing the proportion of precursor cells in the periphery, these results confirm that the precursor cells responsible for allograft rejection are neither recently formed nor short-lived.

Discussion

The mechanism whereby allogeneic tissue is actually destroyed in vivo is unknown. Although a large body of in vitro work strongly implicates a cytotoxic T cell as the most likely effector mechanism in graft rejection this has not been unequivocally shown to operate in the intact animal (23–25). The adoptive transfer assay described here makes it possible to examine the relevance of various cell populations, singly or in combination, to the process of graft rejection. The irradiated heart-grafted assay animal is >99.9% depleted of lymphocytes but contains the somatic structures necessary for the physiological function of the immune system in vivo (7, 8). Such animals support lymphocyte recirculation through normal traffic areas (26), and permit the normal differentiation of precursor cells into both T effector and plasma cells and the synthesis of normal titers of antibody (including alloantibody). It has been firmly established that the immune responses restored to such animals are mediated by the cells adoptively transferred to them (7, 8, 26) and not by a nonspecific stimulatory effect on the animals own immune system.

The results of adoptive restoration of heart graft rejection reported here establish a central role for the long-lived recirculating T lymphocytes in first-set allograft rejection. Not only were grafts rejected with normal tempo in rats restored with highly purified inocula of these cells, but the concomitant production of alloantibody in animals given B cells in addition had no effect on the tempo of rejection. A role for alloantibody has been demonstrated in hyperacute rejection in some species (27–29) and its relevance to normal first-set rejection deduced by inference (6). It has also been suggested that small amounts of locally produced antibody may play a part in graft rejection (30). Our observations indicate that first set rejection can be achieved in the absence of B cells, alloantibodies in detectable titer, or significant plasma cell infiltrate, and strongly suggest that B-cell-mediated responses are ancillary phenomena in the primary rejection of grafts expressing major alloantigens.

The conclusion that the T cells capable of causing rejection are in the recirculating pool rests upon their presence in large numbers in tissues known to be rich in recirculating lymphocytes and the direct demonstration that those cells in these tissues which are responsible for first-set rejection do indeed recirculate rapidly. That the spleen is a poor source of these cells was unexpected in view of previous evidence suggesting that the spleen contains large numbers of recirculating lymphocytes (31, 32). This evidence is however essentially indirect. The finding that large numbers of injected TDL home to the spleen (33) has led to the conclusion that this organ contains large numbers of recirculating lymphocytes (34). When this question was examined directly by testing the ability of spleen cells to recirculate from blood to lymph it was obvious that, though recirculating cells are present in spleen cell populations, they are poorly represented here in comparison with their proportions in lymph nodes and thoracic duct lymph. This can be explained by the much more rapid transit of recirculating cells through the spleen than the lymph nodes (31, 35) resulting in a low instantaneous content of recirculating cells in the former.

The failure to abrogate skin allograft rejection across the MHC by procedures

which deplete the recirculating pool (36, 37) might suggest that the precursors of cells which mediate this response cannot be completely restricted to this pool. However, first-set skin allograft rejection is routinely achieved by adoptive transfer of as few as 3×10^7 lymphocytes (8) which is about 2% of the recirculating pool. Depletion experiments would therefore not be expected to affect skin graft survival unless they were >98% effective, a level which cannot be practically achieved in vivo. In favor of restriction of effector cell precursors to the recirculating pool are the observations recorded in this paper that the small number of rapidly recirculating T cells which can be isolated from spleen cell inocula are equipotent with recirculating cells derived from other sources and that the distribution of effector cell precursors within the lymphoid system parallels the concentration of recirculating lymphocytes in the system. We would therefore conclude that both the necessary and sufficient mediator of first-set allograft rejection is a long-lived, recirculating T cell.

Summary

The nature of the cells required for first-set graft rejection in vivo was examined by using an adoptive transfer system to restore heart-graft rejection in irradiated rats. Highly purified inocula of peripheral T lymphocytes were shown to quantitatively account for the restorative ability of adoptively transferred cells. These T cells were shown to be long-lived small lymphocytes which are not recently derived from the thymus during adult life. They belong to the pool of T cells which constantly recirculate from blood to lymph as shown by their rapid appearance in the lymph of irradiated syngeneic rats after intravenous injection. Neither B lymphocytes nor antibodies in the circulation or in the graft itself are required for first-set graft rejection.

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