

FIDELITY OF THE REPERTOIRE IN T CELL RECONSTITUTED ATHYMIC NUDE RATS

Preservation of a Deficit in Alloresponsiveness Over One Year

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The thymus is essential for the generation of mature T cells, but its role in maintaining the recirculating pool of T lymphocytes has not been clearly established. Too few cells leave the thymus to account for the size of the peripheral T cell pool (1), suggesting that extrathymic T cell proliferation at least partially maintains total T cell numbers. The stimulus for this postthymic T cell division is not known. Stutman and colleague (2, 3) have argued that the thymus exports T cell "progenitors" that divide and differentiate in the periphery and account for the major part of the peripheral T cell pool. Alternatively, mature T cells may expand extrathymically, driven predominantly by exogenous antigen (4). The former mechanism might be expected to maintain a diverse but homonymous repertoire, while a purely antigen-driven system would favor a selective and unequal expansion with a relative loss of diversity.

To investigate postthymic changes we have taken advantage of the observation that peripheral T cells expand and permanently reconstitute athymic nude rats (5). The congenital absence of the thymus that characterizes nude rats and mice is reflected in the complete lack of certain T-dependent responses *in vivo* (6, 7). Early death of the animal ensues unless T cell functions are provided through thymus grafting (8, 9) or by the adoptive transfer of syngeneic mature T cells from euthymic donors (5, 7). The latter procedure donates full immunocompetency after injecting 0.1% or less of the normal rat complement of T cells (5). These small numbers of T cells expand 10–100-fold in the absence of a thymus, persist for >2 yr, and restore graft-versus-host (GVH)¹ responses and the ability to reject allografts (5) and to mount T-dependent antibody responses.

If the initial inoculum of mature T cells has a partial "hole" in its antigenic repertoire, we can ask whether that defect is faithfully preserved during and after the ensuing expansion? The inoculum was obtained from the thoracic ducts of syngeneic rats, but before injection into the athymic nude recipient was passaged from blood to lymph in an irradiated F₁ hybrid intermediate expressing the haplotype of the syngeneic parent. This passage procedure removes the great majority of T cells responsive to the second parent by the process of negative selection (10).

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¹ *Abbreviations used in this paper:* ALC, allogeneic lymphocyte cytotoxicity; GVH, graft-vs.-host; LN, lymph node; TDL, thoracic duct lymphocytes.

We show here that this deficit of alloreactivity in the T cell repertoire, as assessed by the local GVH popliteal lymph node (LN) assay, is maintained in nude recipients, even when tested at intervals up to 13 mo after transfer and after a >15-fold expansion.

Materials and Methods

Rats. The following inbred lines of rats and selected F₁ hybrids were bred and were maintained in the Animal Unit, Manchester University Medical School: PVG, PVG.3T (syngeneic with PVG but carrying a chromosome marker), AO, DA, BN, (DA × PVG)F₁, (AO × PVG)F₁, and (BN × PVG)F₁. A colony of congenitally athymic nude rats (PVG.rnu/rnu) was bred and maintained in conventional animal house conditions as previously described (5). In some instances euthymic heterozygotes (PVG.rnu/+) were used as a source of control thoracic duct lymphocytes (TDL).

Reconstitution of Nude Recipients. Syngeneic PVG TDL were obtained from donors of the PVG.3T strain (11) by the establishment of a thoracic duct fistula (12). TDL were washed twice in PBS containing essential mineral salts (Dulbecco's solution, Oxoid Ltd., Basingstoke, UK) and injected intravenously into nude recipients or alternatively passaged from blood to lymph through intermediate rats as described below. Syngeneic lymphocyte populations were negatively selected (i.e., depleted of specific alloreactivity [10]) by injecting PVG TDL intravenously to 400 rad irradiated, thoracic duct-cannulated F₁ hybrid rats and collecting cells draining from the thoracic duct fistulae during the subsequent 36 h. Passaged TDL were double-negatively selected by similarly passaging TDL from blood to lymph through second intermediate rats. Twice passaged TDL were tested for their (depleted) ability to initiate a local popliteal LN GVH response and were also used to reconstitute PVG nude recipients by injecting 20 × 10⁶ cells intravenously. Details of cells negatively selected by passage twice through (DA × PVG)F₁ intermediates or through two different F₁ hybrid intermediates are summarized in Table I.

Fluorescence Staining. Cells were stained with the following mAbs and analyzed by flow cytometry as previously described (5): W3/25 (anti-CD4), OX8 (anti-CD8), OX19 (anti-CD5), and OX12 (anti-rat κ light chains). Second layer staining was with FITC-anti-mouse IgG extensively absorbed to remove all anti-rat Ig activity. Biotinylated mAbs and the secondary reagent phycoerythrin-conjugated streptavidin were the kind gift of Dr. G. W. Butcher (Babraham, Cambridge, UK): GN7/6 and GN 7/10 (anti-RT1A^a, class I of AO strain), YR5/12 and YR5/310 (anti-RT1A^c, class I of PVG strain).

GVH Activity. GVH activity was measured by a standard parental into F₁ hybrid popliteal LN weight assay (13). Comparisons between and within experiments were made by expressing all responses in terms of R1 values as previously detailed (5). R1 represents the "response" in milligrams of LN weight had a dose of 10⁶ cells been injected into the footpad and is derived from the equation:

$$\log R1 = b (\log 10^6 - \log x) + \log \bar{y} \quad (\text{Eq. 1})$$

where *b* is the common slope for a given F₁ hybrid strain, *x* is the injected cell dose (which may be greater or less than 10⁶), and \bar{y} is the mean LN weight in milligrams derived from three to five individual draining nodes. Mean common slopes (*b*) for the present experiments are: (AO × PVG)F₁, *b* = 0.482; (DA × PVG)F₁, *b* = 0.855; (BN × PVG)F₁, *b* = 0.715.

Irradiation. Rats were given 400 rad whole body irradiation in 20 min from a cobalt-60 source 3 d before use.

Results

Nude Rats Reconstituted with Negatively Selected TDL. TDL from PVG donors were negatively selected by passage through two consecutive (DA × PVG)F₁ recipients. These intermediates were lightly irradiated 3 d before thoracic duct cannulation and cell injection. Details are given in Exp. 1, Table I. The passaged and nonpassaged (np) TDL were tested in local popliteal LN GVH assays and were also used to recon-

TABLE I
*Experimental Protocol for Obtaining Negatively Selected PVG TDL for Transfer to
 Nude Recipients: Summary of Cell Recoveries*

Exp.	TDL output*	1st intermediate [†]		TDL output (36 h)	2nd intermediate [†]		TDL output (36 h)	Designation of TDL injected [§]
		n	Strain		n	Strain		
1	63 × 10 ⁸	4	(DA × PVG)F ₁	12.5 × 10 ⁸	2	(DA × PVG)F ₁	6.35 × 10 ⁸	(DA→DA)TDL PVGnpTDL
	2 × 10 ⁸		Not passaged					
3	21 × 10 ⁸	1	PVG	6.8 × 10 ⁸	1	(DA × PVG)F ₁	1.46 × 10 ⁸	(PVG→DA)TDL
	29 × 10 ⁸	1	(AO × PVG)F ₁	7.5 × 10 ⁸	1	(DA × PVG)F ₁	4.0 × 10 ⁸	(AO→DA)TDL
	2 × 10 ⁸		Not passaged					PVGnpTDL

* TDL obtained from 20 (Exp. 1) and 18 (Exp. 3) PVG donors.

[†] Rats irradiated with 400 rad 3 d before use. TDL output from noninjected irradiated (DA × PVG)F₁ rats was 0.45 × 10⁸ cells in 36 h.

[§] Each nude injected with 20 × 10⁶ TDL.

stitute PVG.rnu/rnu rats. Nude recipients were examined for GVH activity against specific (DA × PVG)F₁ (anti-DA) and (AO × PVG)F₁ third-party (anti-AO) alloantigens. The response of PVGnpTDL shows the expected GVH-induced LN weight increase with increasing doses of cells when tested for anti-DA and anti-AO alloreactivity (Fig. 1, *A*, *B*). In contrast, PVG TDL passaged twice through (DA × PVG)F₁ rats were depleted of anti-DA activity (Fig. 1 *A*), but not of anti-AO responsiveness (Fig. 1 *B*).

LN cells or TDL from nude rats reconstituted with negatively selected TDL 8 mo and 12 mo earlier, respectively, were similarly tested for GVH activity. Cells from recipients of negatively selected TDL showed little activity to specific (DA) alloantigens (Fig. 1, *C* and *E*), but responded as well to third-party (AO) alloantigens (Fig. 1, *D* and *F*) as did cells from recipients of PVGnpTDL.

For ease of comparison, these and subsequent data were converted as follows. Cells obtained from each nude recipient were tested at more than one cell dose whenever possible. The response for a given cell dose injected into an F₁ footpad was derived from the geometric mean of three to five draining popliteal LN. To allow comparisons and statistical evaluation we converted the results to mean R1 values (see Materials and Methods), i.e., the calculated LN weight that would be produced by 10⁶ cells. Using R1 values, the activity of a given cell population (the numerator) was expressed as a ratio (response ratio) relative to the R1 response of cells obtained from 8-mo nude recipients of PVGnpTDL (the denominator).

When expressed in this way (Table II), additional information can be derived from the results in Fig. 1. The starting population of TDL, tested at the time of transfer to nude recipients, was apparently more responsive than the cells recovered 8 mo or 1 yr later. This finding is consistent with our earlier report (5). The passage of PVG TDL through (DA × PVG)F₁ intermediates reduced the specific response to DA alloantigens by 50-fold but did not affect the response to third-party AO alloantigens. At 8 mo and 1 yr after transfer to nude recipients, the activity of passaged TDL to DA alloantigens remained 5–10-fold lower than that of nonpassaged TDL. Again, the response to third-party alloantigens was unchanged.

Co-transfer of F₁ Hybrid Cells. TDL obtained after passage through lightly irradiated F₁ hybrid intermediates include a small number (7–15%) of contaminating F₁

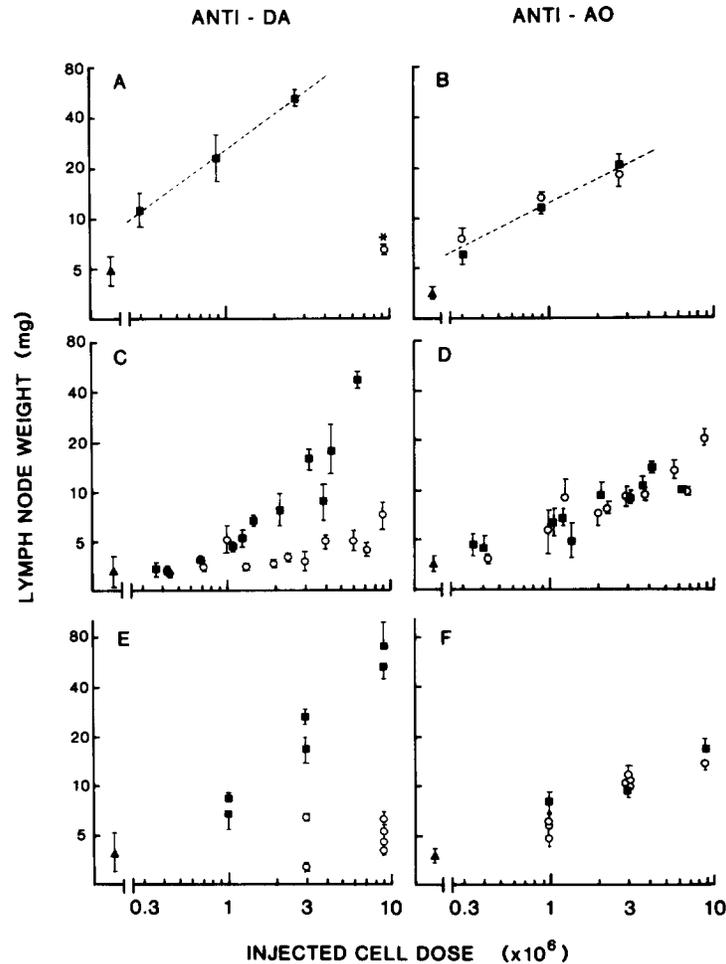


FIGURE 1. The response of PVG TDL (*A, B, E, F*) or PVG LN cells (*C, D*) to (DA \times PVG) F_1 (anti-DA) and (AO \times PVG) F_1 (anti-AO) alloantigens as assayed by the local GVH popliteal LN weight assay. Nonpassaged PVGnpTDL (■) and (DA \rightarrow DA)TDL negatively selected by passage from blood to lymph through two consecutive (DA \times PVG) F_1 intermediates (○) were tested in specific (anti-DA) and third-party (anti-AO) F_1 hybrids; (▲) control responses after PBS injection; (*) 20×10^6 (DA \rightarrow DA) TDL injected into footpads. Cells were tested before transfer (*A, B*) to PVG nude recipients, or were recovered from nude recipients and tested 8 mo (*C, D*) and 12 mo (*E, F*) after transfer. A total of 16 reconstituted nudes were investigated, 8 receiving PVGnpTDL, 8 receiving negatively selected (DA \rightarrow DA)TDL equally divided between the 8 and 12 mo experiment. Each point is the geometric mean \pm SE of three to six popliteal LN weights.

cells. We asked whether the persistent deficit of alloresponsiveness observed in the above experiment (Fig. 1, Table II), depended on the concomitant induction of tolerance by co-transfer of the F_1 cells. To test this hypothesis, 20×10^6 PVGnpTDL were transferred to nude recipients with or without TDL (7.8×10^6) from irradiated (AO \times PVG) F_1 hybrids. GVH activity was assessed using LN cells obtained from these recipients 9 mo later. The addition of F_1 cells from irradiated donors did not reduce the GVH activity (Table III). As a further test for a "tolerance-inducing"

TABLE II
GVH Activity of Negatively Selected TDL Recovered from
Nude Recipients 8 and 12 mo after Transfer

Source of cells*	R1 values [†]		Response ratios	
	Anti-DA	Anti-AO	Anti-DA	Anti-AO
Before transfer				
PVGnpTDL	26.31	11.28	5.18	2.02
(DA→DA)TDL	0.51	12.93	0.100	2.31
8 mo after transfer				
PVGnpTDL	5.08	5.59	1.00 [§]	1.00 [§]
(DA→DA)TDL	1.14	5.62	0.224	1.01
12 mo after transfer				
PVGnpTDL	8.12	6.54	1.60	1.17
(DA→DA)TDL [¶]	0.770	5.41	0.152	0.968

* See Table I, Exp. 1 for details.

† Means derived from the data in Fig. 1.

§ Assigned a value of unity as a basis for comparison.

|| FACS analysis of TDL from four nude recipients of PVG(np): 34.5%, W3/25⁺; 5.3%, OX8⁺; 41.1%, OX19⁺.

¶ FACS analysis of TDL from two nude recipients of DA→DA: 26.4%, W3/25⁺; 5.4%, OX8⁺; 30.7%, OX19⁺.

mechanism, PVG TDL were passaged through (AO×PVG)F₁ intermediates, but collection was delayed until the time when alloresponsive cells are released from tissues and return to the recirculating pool as blasts (14). If tested at this stage, the blasts are inactive in a GVH assay but recover their activity with time (15). Therefore, the late collection of passaged TDL would contain both alloresponsive cells and F₁ contaminants. 9 mo later, LN cells from recipients of late passaged TDL had GVH

TABLE III
Co-transfer of F₁ Hybrid TDL with PVG TDL Does Not Induce GVH
Tolerance in Lymphocytes Recovered from Nude Recipients 9 mo Later

Source of cells*	Additional cells [†]	R1 values [§]		Response ratio	
		Anti-AO	Anti-DA	Anti-AO	Anti-DA
Pre-transfer					
PVGnpTDL	NA	ND	13.25	NA	1.92
Nude recipient 9 mo after transfer of:					
PVGnpTDL	Nil	6.53	6.90	1.00	1.00
PVGnpTDL	(AO × PVG)F ₁	7.38	9.22	1.13	1.34
late TDL	Nil	5.05	11.18	0.77	1.62

* PVGnpTDL from euthymic PVG.rnu/+ donors; late TDL obtained from (AO × PVG)F₁ intermediate rat 45–71 h after intravenous injection of 2 × 10⁹ PVG TDL. TDL recovered during this late period contain anti-AO-responsive cells released from tissues.

† Obtained from irradiated (AO × PVG)F₁ rats. 7.6 × 10⁶ cells transferred with 20 × 10⁶ TDL (np) to nude recipients.

§ Means derived from three to six F₁ popliteal LN weights per cell suspension.

|| LN cells analyzed in GVH LN assays; three, three, and two nude recipients tested in each group, respectively.

activity not significantly different from PVGnpTDL (Table III), showing that tolerance was not induced.

An attempt was made to deplete passaged TDL of contaminating F₁ cells by treatment with appropriate alloantisera. Nude recipients of TDL that had been passaged through (AO × PVG)F₁ intermediates and then treated with anti-AO antisera did not survive long enough for analysis, for reasons that are not understood.

Nude Rats Reconstituted with Double-negatively Selected TDL. Removal of contaminating F₁ cells was achieved by an alternative approach making use of the fact that irradiated F₁ cells recirculate poorly and of a phenomenon known as allogeneic lymphocyte cytotoxicity (ALC) (16). Allogeneic lymphocytes injected intravenously are rapidly killed (cell destruction begins within 15–30 min) in both euthymic and nude recipients by a radioresistant non-T cell, non-B cell, non-alloantibody-mediated mechanism (17). Cytotoxic effector cells in the host recognize target antigens on allogeneic lymphocytes coded by genes within the MHC (18, 19). In the following experiments (Exp. 3, Table I) PVG TDL were passaged initially through (AO × PVG)F₁ intermediates and then through (DA × PVG)F₁ intermediates before transfer to nude recipients. Contaminating (AO × PVG)F₁ cells would be removed by the second allogeneic (DA × PVG)F₁ intermediate. In addition, the resulting population should also be negatively selected to both AO and DA alloantigens.

Phenotypic analysis of control PVG TDL passaged through syngeneic PVG followed by (DA × PVG)F₁ intermediates (PVG → DA) or PVG TDL passaged through (AO × PVG)F₁ followed by (DA × PVG)F₁ intermediates (AO → DA) is shown in Table IV. As expected, because T cells recirculate faster than B cells (20), passaged TDL were enriched for W3/25⁺ (CD4) T cells (67–72% vs. 35–40% W3/25⁺ in PVGnpTDL). Using mAbs against the class I MHC of AO (anti-RT1A^u), 7% of control PVGnpTDL (RT1^c) stained positively compared with 7.7% of AO → DA passaged TDL, indicating that there were few if any contaminating (AO × PVG)F₁ cells in the inocula given to nude recipients.

The GVH activity of PVGnpTDL and double passaged TDL was tested before transfer, 4 mo after transfer, and finally in surviving animals 13 mo after transfer to nude rats. LN weights obtained from GVH assays were converted to R1 values and response ratios as before. Sequential passage through two different intermediates (TDL AO → DA) effectively depleted TDL of specific activity to both alloantigens (Table V). Nude recipients of these double-negatively selected TDL were examined

TABLE IV
*Phenotypic Analysis of Passaged and Nonpassaged (np)
PVG TDL and TDL from F₁ Hybrids*

Source of cells	Mix of RT1 allotypes	Percentage positive for					
		W3/25	OX8	OX19	OX12	RT1A ^u	RT1A ^c
PVGnpTDL	c	—	—	—	—	7.0	94.5
(DA × PVG)F ₁ npTDL	(a × c)	—	—	—	—	5.4	91.7
(PVG → DA)TDL*	c + (a × c)	72.3	3.4	82.2	0.1	5.3	87.8
(AO × PVG)F ₁ npTDL	(u × c)	—	—	—	—	86.8	94.7
(AO → DA)TDL*	c + (a × c) + (u × c)	67.1	9.7	77.0	2.3	7.7	80.6

* PVG TDL twice passaged. See Table I, Exp. 3, for details.

TABLE V
*GVH Activity of Double-negatively Selected PVG TDL Tested before Transfer
 and Tested on Recovery from LN 4 mo after Transfer to PVG Nude Recipients*

Source of cells*	R1 values [‡]			Response ratio		
	Anti-AO	Anti-DA	Anti-BN	Anti-AO	Anti-DA	Anti-BN
Before transfer						
PVGnpTDL	7.14	24.3	ND	1.0	1.0	NA
(PVG→DA)TDL	12.90	ND	ND	1.81	NA	NA
(AO→DA)TDL	2.40	1.11	ND	0.392	0.046	NA
4 mo after transfer						
Euthymic TDL [§]	21.0	14.3	25.0	2.08	2.32	2.29
LN cells from nudes injected with:						
PVGnpTDL	10.10	6.15	10.90	1.00	1.00	1.00
(PVG→DA)TDL	7.41	1.35	8.32	0.734	0.220	0.763
(AO→DA)TDL	3.60	1.86	7.89	0.357	0.302	0.724

* See Table I, Exp. 3, for details.

[‡] Means derived from three to six F₁ popliteal LN weights.

[§] Obtained from fresh donors at the time of 4 mo GVH assay.

^{||} Three nude recipients tested in each group.

4 mo later. LN cells from nude recipients remained negatively selected to both AO and DA alloantigens (ratios of 0.357 and 0.302, respectively). When tested against third-party (BN×PVG)F₁ alloantigens, the response (ratio 0.724) was not significantly different from that induced by PVGnpTDL. The use of control TDL (PVG → DA) confirmed the specificity of the negative selection and showed that the deficit in the T cell repertoire was limited to alloantigens of the DA haplotype (ratio 0.220).

13 mo after injection, two nudes receiving double-negatively selected TDL and one receiving PVGnpTDL were examined (Table VI). A comparison of the response ratios shows that the response to AO and DA alloantigens had not returned (ratios 0.339 and 0.394, respectively). Since contaminating (AO×PVG)F₁ cells were removed by the second passage, the results cannot be explained by tolerance induction. There was no evidence that the deficit or hole in the T cell repertoire was restored by cells derived from either the donor or the host.

TABLE VI
*GVH Activity (R1 values) of Double-negatively Selected TDL
 Recovered from LN of Nude Recipients 13 mo after Transfer*

Source of cells*	Anti-AO	Anti-DA	Anti-BN
PVGnpTDL	14.56 [‡]	6.87	15.30
(PVG→DA)TDL	7.23	2.29	19.05
(AO→DA)TDL	3.69	2.51	6.89
Response ratio (mean)	0.339	0.349	0.848

* See Table I, Exp. 3 for details.

[‡] R1 values are derived from three to five F₁ popliteal LN. Single rats examined.

We conclude that fully mature, thymus-derived T cells with the ability to recirculate from blood to lymph are able to reconstitute the peripheral T cell pool of nude recipients. Furthermore, the T cell repertoire of reconstituted nude rats depends entirely on the TCR specificities of the donor population. The specificity of mature T cell populations is faithfully maintained for >1 yr.

Discussion

Earlier work (5) showed that athymic nude rats were reconstituted with T cells after the intravenous injection of 20×10^6 TDL ($\sim 4\%$ of the recirculating pool) from euthymic donors. Numerically, the size of the peripheral T cell pool increased within 2 mo to $\sim 67\%$ of that of euthymic animals. This represented a 10–15-fold expansion. If fewer cells were injected (1.6×10^6), the factor of expansion was greater (80–100-fold) (Bell, E. B., S. M. Sparshott, M. T. Drayson, and S. V. Hunt, manuscript submitted for publication). The ability to reject skin allografts returned immediately and was permanently maintained (5). Allograft rejection depended on CD4⁺ cells alone (Whitby, E. H., S. M. Sparshott, and E. B. Bell, manuscript submitted for publication) and was dose dependent at low doses ($0.1\text{--}5 \times 10^6$ cells). GVH responsiveness returned more slowly; levels of GVH activity that approximated normal were not found until 6–9 mo after reconstitution (5). Our recent work has suggested that the somewhat reduced GVH response in reconstituted nude rats could be explained by an altered ratio of surviving CD4⁺ T cell subsets (unpublished observations) defined in the rat (21, 22) by the mAb OX22 (anti-CD45R).

The present studies conclusively demonstrate that mature, recirculating T cells passaged from blood to lymph of two consecutive intermediates (from which immature precursors or progenitors were presumably excluded), were entirely successful at reconstituting nude rats. In addition, the investigation showed that a defect in the repertoire of transferred T cells was faithfully maintained in nude recipients 4–13 mo later. Other investigations (Bell, E. B., M. T. Drayson and C. S. Ramsden, unpublished observations) demonstrated that TDL negatively selected to soluble protein antigens, which failed to induce a thymus-dependent antibody response to these specific antigens, also remained unresponsive in nude recipients 6 mo later. The most likely explanation of these findings is that the T cells recovered many months later in nude recipients were the progeny of the precommitted donor population. Using allotype marked TDL we have directly demonstrated, both phenotypically and functionally, that the only T cells recovered from reconstituted nude rats up to 2 yr later were donor derived (Bell, E. B., et al., submitted for publication). Our studies were concerned primarily with CD4⁺ T cells. We have limited quantitative information on CD8⁺ T cells.

Whether nude animals develop CD4⁺ T cells with time but without the benefit of the thymus has been discussed in detail elsewhere (23, 24). Even with the increased lifespan afforded by injection of TDL, we have found no evidence that nude rats develop their own CD4⁺ T cells. Recently it was shown that athymic CD4⁺ cells were defective and unable to proliferate (25). It has been suggested that nude-derived T cells arise and expand oligoclonally (26), which poses obvious difficulties in demonstrating reactivity to specific antigens. The high frequency of allospecific T cells (4–12%, references 27, 28) makes the alloantigen system the one most likely to uncover func-

tional nude-derived T cells, particularly if they only arise in an oligoclonal fashion. There is no indication in the present or previous (Bell, E. B., et al., submitted for publication) investigation that nude rats develop extrathymic T cells that are able to respond to alloantigens.

The transfer of negatively selected TDL into T cell-deficient recipients is reminiscent of earlier studies (15, 29) in which positively selected T cells were "parked" in B animals (thymectomized, irradiated, bone marrow restored). T cells were selectively stimulated in vivo using bulk MLRs or in vitro by collecting TDL blasts from F₁ hybrids injected with parental strain T cells. The T blasts were transferred to B animals and allowed to recover. 4–6 wk later TDL from restored B animals were tested in MLR and GVH assays. Consistent with the present study, the specificity of positively selected T cells was retained during this relatively short period.

Cell proliferation within the thymus is extensive (1, 30). Paradoxically, though, the number of T cells that exit is not sufficient to establish or even maintain the peripheral T cell pool at its known level (1, 30). To account for this discrepancy, Stutman (2, 31) proposed that the thymus also exports thymus "progenitors" that differentiate and divide extensively in the periphery (3), independently of antigen. Others have suggested that postthymic T cells self-renew extrathymically (32–34) by a mechanism based on exogenous antigen encounter (32). Our studies do not discount the contribution of a thymus-derived T cell "progenitor" in euthymic animals, but clearly show that in its absence mature T cells can self-renew; it is not necessary to hypothesize a postthymic progenitor to resolve the paradox of low thymus output.

The deficit in alloreactivity in negatively selected TDL after double passage was >90%, but not absolute. It is intriguing that the many-fold expansion of the donor population and its continued self-renewal for >1 yr did not generate a correction of the deficit by preferential expansion of the few remaining allospecific T cells. This finding is not easy to reconcile with an antiidiotypic network system of regulation. Negative selection would presumably not deplete antiidiotypic clones. According to this theory, antiidiotypic cells should drive expansion of the few specific T cells that escaped depletion and with time eliminate the deficit. If the extrathymic maintenance of the T cell pool was to be entirely dependent on exogenous antigen, one would expect the T cell repertoire to be perturbed by specific antigen encounter. Certainly repeated stimulation of newly reconstituted nude rats by a specific alloantigen can lead to a selective increase in activity to that alloantigen (Drayson, M. T., and E. B. Bell, unpublished observations).

A mechanism of extrathymic expansion not directly dependent on exogenous antigen should not be wholly excluded. There is no conceptual difficulty in, for example, proposing a bystander expansion of T cells by IL-2 or IL-4 in the microenvironment. Such a phenomenon might occur in any lymphoid tissue since the expansion of small numbers of syngeneic T cells occurs just as well in splenectomized nude rats (our unpublished observations). The periarteriolar lymphatic sheath of spleen and the paracortex of LN are the hubs of T lymphocyte traffic. These areas are also sites enriched with interdigitating (dendritic) cells proficient in stimulating T cell division. A mechanism of this type could maintain not only the size of the T cell pool but also its original diversity.

The reconstitution of immunodeficient animals with T cells that we (5) and others (2, 3, 32, 34) have observed illustrates an important homeostatic control mecha-

nism. The fact that mature committed T cells expanded in peripheral lymphoid tissues and were permanently maintained at a stable level indicates that the size and quantity of the peripheral T cell pool can be regulated outside the thymus (at least in the nude rat). We see no reason to suppose that control of the postthymic T cell pool in euthymic animals is any different. The presence of a thymus would, of course, provide a continuous topping up of the repertoire; as demonstrated here, the repertoire of T cells that have already emerged from the thymus is committed. It would appear that the thymus is primarily concerned with maintaining the quality of the T cell pool rather than the quantity.

Summary

A single intravenous injection of a relatively small number of T cells contained in the population of rat thoracic duct lymphocytes (TDL) is sufficient to restore to normal the peripheral T cell pool of athymic PVG.rnu/rnu nude rats. The donor T cells expand >10-15-fold, self-renew, and restore immunocompetency to nude recipients permanently (>2 yr). We asked whether the T cell repertoire was affected by the expansion and self-renewal process. Nude recipients were injected with syngeneic PVG TDL that had been allospecifically depleted (negatively selected) by consecutive passage from blood to thoracic duct lymph through two irradiated (DAxPVG) F_1 intermediate rats. Negatively selected TDL were tested before transfer by the $P \rightarrow F_1$ popliteal LN GVH assay and showed a >90% depletion of specific reactivity to DA alloantigens. Surviving cells or their progeny were recovered from LN or TDL of nude recipients 8 and 12 mo after transfer. The deficit in GVH reactivity to the DA haplotype persisted, but normal GVH activity was demonstrated against a third party (AOxPVG) F_1 alloantigen. The "hole" in the repertoire could not be attributed to tolerance induced by the co-transfer of contaminating irradiated F_1 TDL. PVG TDL passaged consecutively through (AOxPVG) F_1 and (DAxPVG) F_1 intermediates and devoid of (AOxPVG) F_1 cells remained specifically depleted to both AO and DA haplotypes when recovered from nude recipients 4 and 13 mo later, but displayed GVH activity to a third-party (BNxPVG) F_1 alloantigen. Thus the exact specificity of the T cell repertoire of the original inoculum was faithfully maintained in nude recipients throughout the initial phase of rapid expansion and the continued self-renewal of the mature peripheral T cell pool.

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