

Thymus-grafted SCID Mice Show Transient Thymopoiesis and Limited Depletion of $V\beta 11^+$ T Cells

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Summary

To seek direct evidence for the notion that stem cells in the thymus need to be constantly replenished from the bone marrow (BM), fetal (day 15) thymuses from normal BALB/c mice were grafted into T and B cell-deficient C.B-17 SCID mice (both H-2^d, I-E⁺). The thymus grafts in these mice showed normal thymopoiesis for the first 3 wk postgrafting but then developed sudden atrophy with near complete loss of CD4⁺8⁺ cells by 4–5 wk. Such atrophy was not seen when the thymus-grafted mice were cotransplanted with normal BM cells. The lymph nodes of SCID mice receiving thymus grafts alone contained mature T cells but virtually no B cells. This lack of B cells was associated with aberrant I-E-restricted $V\beta$ deletion: the depletion of $V\beta 3^+$ and $V\beta 5^+$ T cells was near complete, whereas $V\beta 11^+$ cells showed only marginal depletion.

Neonatal thymus grafts (TG)¹ placed in normal or thymectomized mice undergo considerable enlargement and remain functional for prolonged periods (1, 2). The cells proliferating in the TG are initially of donor origin but are rapidly replaced by host-derived cells. This transition is complete by 1 mo posttransfer. Similarly, intrathymic injection of CD4⁺8⁺ thymic stem cells results in only transient thymopoiesis by donor-derived cells (3–5). These data imply that the self-renewing capacity of thymic stem cells is limited and that maintenance of thymic function depends on a continuous inflow of stem cells from the bone marrow (BM).

A corollary to this notion is that depriving the thymus of an exogenous source of functional stem cells would lead to rapid atrophy. This prediction can be tested by placing TG in SCID mice (6). These mice have an autosomal recessive mutation that prevents the formation of T and B cells; T cell differentiation in SCID mice proceeds only to the stage of CD4⁺8⁺ thymocytes. We show here that day 15 BALB/c fetal TG placed in H-2-compatible adult C.B-17 SCID mice exhibit normal thymopoiesis for 3 wk but then undergo sudden atrophy with selective disappearance of CD4⁺8⁺ cells. The LN of these mice contain large numbers of T cells but virtually no B cells. The absence of B cells is associated with aberrant $V\beta$ deletion.

Materials and Methods

Mice. C.B-17 SCID mice (6) and other mice were obtained from the breeding colony of the Scripps Research Institute. SCID mice were maintained under pathogen-free conditions. Note that C.B-17 and BALB/c are identical except for an Ig allotype difference.

Thymus Grafting. Day 15 fetal thymuses from timed-pregnant mice were inserted under the kidney capsule (four lobes/recipient) of young adult SCID mice using standard procedures. Some of the grafted mice received $\sim 5 \times 10^6$ T cell-depleted normal donor BM cells (7) intravenously within 1 d of grafting.

Flow Cytometry. Thymus and/or LN cells were stained for expression of CD4 (GK1.5), CD8 (53-5.7, 3.168, or YTS 169), α/β TCR (H57-597), IL-2R (7D4), B220 (14.8), CD45RB (23G2), Thy-1.1 (19E12), and TCR $V\beta 3$ (KJ25), -5 (MR9.4), -8 (F23.2), and -11 (RR3-15), as described elsewhere (7–10), using two- or three-color immunofluorescence and analysis on a FACScan[®] flow cytometer (Becton Dickinson & Co., Mountain View, CA). Briefly, cells were double stained for CD4 and CD8 using PE-conjugated anti-CD4 mAb plus biotinylated anti-CD8 mAb followed by FITC-conjugated streptavidin (Zymed Laboratories, San Francisco, CA). For other markers, cells were stained with unconjugated hamster (anti- α/β TCR), or rat (anti-IL-2R, anti-B220, anti-CD45RB) mAbs followed by FITC anti-hamster or -rat IgG reagents (Jackson ImmunoResearch, West Grove, PA), rat serum, and PE-conjugated anti-CD4 and/or anti-CD8 mAbs. For TCR $V\beta$ staining, biotinylated mAbs were used with PE-conjugated anti-CD4 mAb plus FITC-streptavidin. For three-color analysis, cells were stained with biotinylated 19E12 or H57-597 followed by FITC-streptavidin plus PE-conjugated anti-CD4 and red 613-conjugated anti-CD8 (Bethesda Research Laboratories, Gaithersburg, MD). Dead cells were excluded by using propidium iodide.

¹ Abbreviations used in this paper: B/c, BALB/c; TG, thymus grafts or thymus grafted.

Results and Discussion

Thymus Grafting. Adult C.B-17 (Thy-1.2) SCID mice were grafted under the kidney capsule with day 15 fetal thymuses taken from BALB/c (B/c) or (B6-Thy-1.1 × B/c)F₁ embryos. When normal donor BM cells (T depleted) were injected intravenously at the time of grafting, the TG increased rapidly in size and remained enlarged for ≥15 wk (Fig. 1, *a* and *b*, and data not shown). The distribution of the four thymocyte subsets defined by CD4 and CD8 expression was normal (Fig. 2 *f*). Injecting BM cells also caused enlargement of the endogenous host thymus and led to the appearance of B cells in the periphery.

When SCID mice were given TG without BM cells, the TG were obviously enlarged at 1–3 wk posttransfer and reached a peak cellularity of $\sim 2 \times 10^7$ cells at 3 wk (Fig. 1 *a*). During this time most of the cells in the grafts were typical CD4⁺8⁺ cells (Figs. 1 *b*, and 2, *b* and *c*). Single-positive CD4⁺8⁻ and CD4⁻8⁺ cells were rare at 1 wk but reached normal levels at 2–3 wk; TCR expression was normal, with small numbers of TCR⁻ and TCR^{hi} cells and large numbers of TCR^{lo} cells (Fig. 3 *b*). LN of the TG mice were very small at 1 wk but then rapidly enlarged; nearly all of the cells in LN were T cells (Figs. 1 *a*, and 2, *k*, *l*, and *m*), and most expressed a virgin/resting (CD45RB⁺) phenotype (Fig. 2 *o*). Significantly, thymopoiesis in the TG ceased abruptly after 3 wk; marked atrophy ensued and cell counts decreased 20-fold (Fig. 1 *a*). The proportion of CD4⁺8⁺ cells in the grafts fell sharply and these cells were almost undetectable after 4 wk (Figs. 1 *b*, and 2, *d* and *e*). At ≥4 wk, the few cells recovered from the grafts ($\sim 10^6$ cells) consisted of discrete subsets of TCR⁻ and TCR^{hi} cells, with TCR^{lo} cells being conspicuously absent (Fig. 3 *a*, compare with *b*). The TCR^{hi} cells comprised a mixture of CD4⁺8⁻ and CD4⁻8⁺ cells with almost no CD4⁺8⁺ cells (Fig. 3 *f*).

Since thymopoiesis in the TG persisted for ≥15 wk in hosts supplemented with donor BM (see above), the dramatic decline in thymopoiesis seen in hosts given TG without BM (seen in four of five mice tested at 4 wk and nine of nine mice tested at 6–15 wk) provides direct support for the long standing notion that the self-renewing capacity of thymic stem cells is limited and that normal thymopoiesis depends upon a constant influx of new stem cells from the marrow (see Introduction).

Two unexpected findings in the TG mice require comment. First, the grafts did not become depleted of mature CD4⁺8⁻ and CD4⁻8⁺ cells. These cells remained in the grafts indefinitely (>15 wk) and, after 4 wk, showed a curious predominance of CD8⁺ cells over CD4⁺ cells (Fig. 2, *d* and *e*). Since CD8⁺ and CD4⁺ cells (but not CD4⁺8⁺ cells) also appeared in the endogenous thymus (Fig. 2 *g*, compare with *a*), we suspect that the presence of these mature T cells reflected immigration from the peripheral lymphoid tissues (after initial release from the TG). In this respect, we have found that the SCID thymus is abnormally permeable to circulating T cells and that, in marked contrast to the normal thymus (10), the SCID thymus rapidly accumulates mature T cells, especially CD8⁺ cells, after intravenous injection of normal LN cells (C. D. Surh and J. S. Sprent, unpublished data). Second, it is notable that, at ≥4 wk postgrafting, the TG contained a high proportion (up to 50%) of CD4⁻8⁻ cells (Fig. 2, *d* and *e*). As in the endogenous SCID thymus (Fig. 2 *h*), most of these CD4⁻8⁻ cells were IL-2R⁺ (Fig. 2 *j*). Grafting with Thy-1-marked thymuses indicated that the late-appearing CD4⁻8⁻ cells in the TG were almost entirely of host (SCID) origin (Fig. 3, *d*, *g*, and *h*), implying that the TG were eventually permeated by substantial numbers of defective SCID stem cells; these cells remained CD4⁻8⁻ (Fig. 3 *g*).

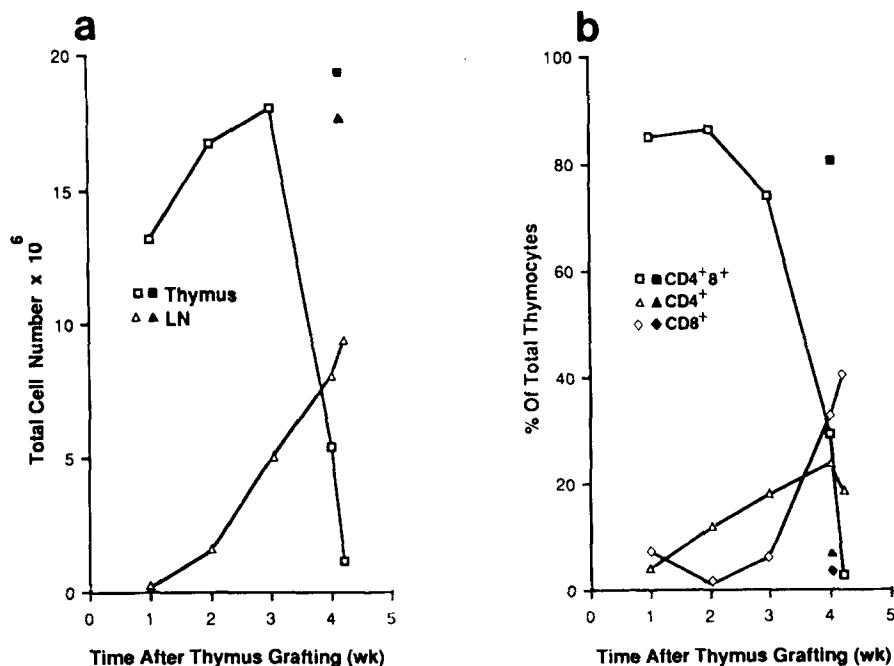


Figure 1. Features of thymus and LN cells recovered from SCID mice receiving BALB/c TG alone (open symbols) or TG plus BM cells (filled symbols). (a) Total viable cells in suspensions prepared from the TG (□ and ■) or from pooled inguinal, brachial, axillary, and mesenteric LN (△ and ▲). (b) Percentage of CD4⁺8⁺ (□ and ■), CD4⁺8⁻ (△ and ▲), and CD4⁻8⁺ (◇ and ◆) cells in suspensions prepared from the TG; thymocytes were typed for CD4 and CD8 expression as described in Materials and Methods. Each data point is the mean value obtained from two to three mice; each mouse was tested individually.

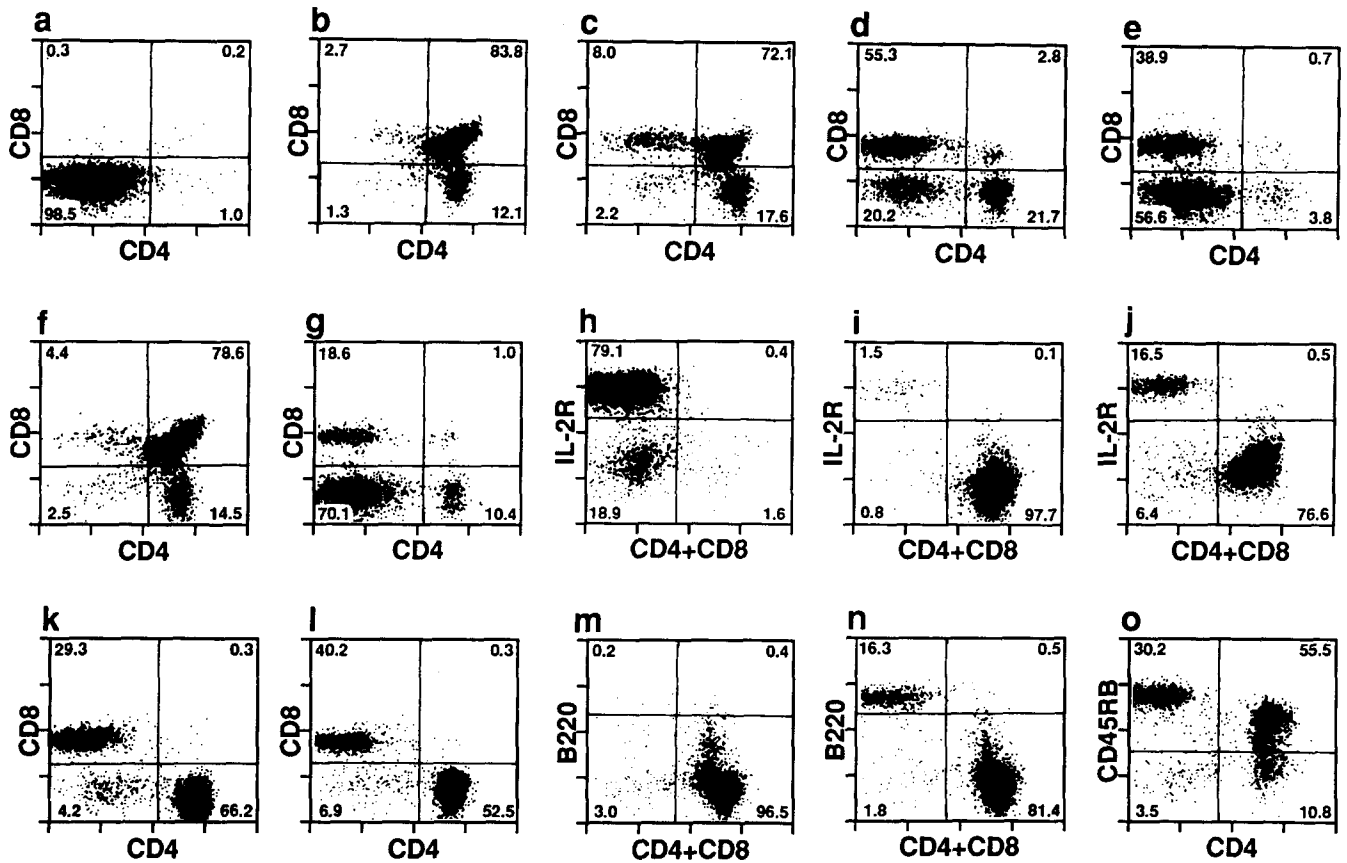


Figure 2. Cell types recovered from SCID mice given BALB/c TG. (a) Cells from normal SCID thymus. (b-e) Cells from TG of SCID mice given TG (without BM) 2 wk (b), 3 wk (c), 4.3 wk (d), or 10 wk (e) before. (f) Cells from TG of SCID mice given TG plus BM cells 10 wk before. (g) Cells from endogenous (host) thymus of SCID mice given TG (without BM) 10 wk before. (h) Same cells as for a. (i) same cells as for c. (j) Same cells as for d. (k) LN cells from SCID mice given TG (without BM) 4.3 wk before. (l) LN cells from SCID mice given TG (without BM) 10 wk before. (m) Same cells as for k. (n) Normal BALB/c LN cells. (o) Same cells as for k. The data shown are representative of two or more mice tested; see Materials and Methods for details of staining.

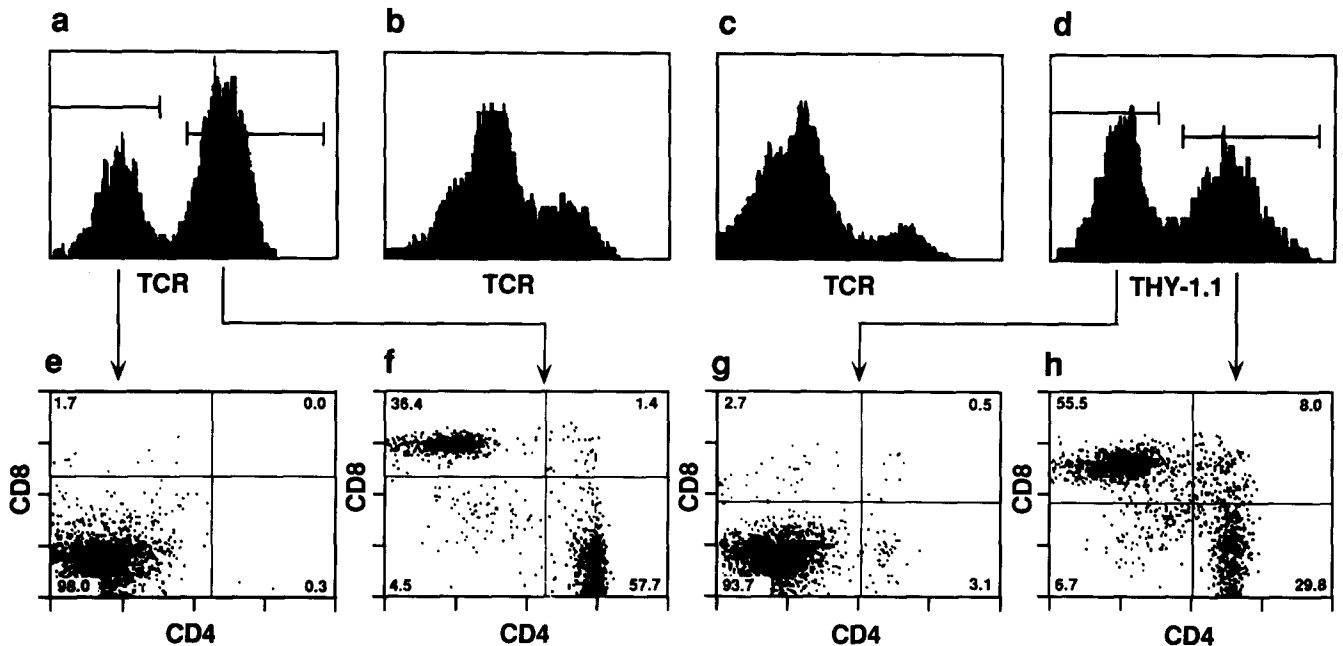


Figure 3. Host origin of CD4⁻CD8⁻ thymocytes recovered from TG of SCID mice given (B6-Thy-1.1 × B/c)F₁ fetal thymuses (without BM cells). Cells were three-color stained for α/β TCR, CD4, and CD8 as described in Materials and Methods. (a) TCR expression on TG thymocytes taken at 8 wk postgrafting; (b) TCR expression on TG thymocytes taken at 2 wk postgrafting; (c) TCR expression on normal BALB/c thymus; (d) Thy-1.1 expression on TG thymocytes taken at 4 wk postgrafting; (e) CD4 vs. CD8 expression on TCR⁻ population in a; (f) CD4 vs. CD8 expression on TCR^{hi} population in a; (g) CD4 vs. CD8 expression on Thy-1.1⁻ (host) population in d; (h) CD4 vs. CD8 expression on Thy-1.1⁺ (donor) population in d.

Table 1. *Vβ* Expression by CD4⁺ Cells from SCID Mice Given Fetal Thymus Grafts ± Bone Marrow Cells

Exp.	Mice tested	Time after thymus grafting	No. of mice tested	Cells tested	Percent of CD4 ⁺ cells expressing:			
					Vβ8	Vβ3	Vβ5	Vβ11
		<i>wk</i>						
1	Normal BALB.B (I-E ⁻)	-	3	LN	19.8	1.48	2.37	4.76
	Normal BALB/c (I-E ⁺)	-	3	LN	19.0	0.10	0.13	1.21
	BALB/c TG + BM → SCID	15	2	LN	16.9	0.00	0.21	1.04
	BALB/c TG → SCID	4	2	LN	18.3	0.19	0.25	4.13
		6	2	LN	NT	NT	0.21	4.42
		10	2	LN	16.7	0.05	0.31	3.98
		15	2	LN	NT	NT	0.05	2.56
2	Normal (B6-Thy-1.1 × B/c)F ₁	-	2	Thy	18.4	0.13	0.13	0.44
	(B6-Thy-1.1 × B/c)F ₁ TG + BM → SCID	8	2	TG	14.7	0.29	0.16	1.10
	(B6-Thy-1.1 × B/c)F ₁ TG → SCID	4	2	TG	6.4	0.16	0.47	5.65
		8	2	TG	14.1	0.17	0.24	3.70

LN cells and thymocytes were stained for CD4 expression vs. Vβ expression using dual fluorescence and flow cytometry (see Materials and Methods). The data on thymocytes refer to mature (Jld⁻) cells (10).

Vβ Deletion. Two groups have shown that Vβ deletion in I-E⁺ mice is less marked in μ-suppressed mice than in normal mice (11, 12). This finding suggests that Vβ deletion is at least partly under the control of B cells. The extreme paucity of B (B220⁺) cells in TG SCID mice (≤0.2% of LN cells; Fig. 2 *m*) makes these mice a useful tool for examining the requirements for I-E-controlled Vβ deletion. As shown in Table 1, Exp. 1, TG SCID mice reconstituted with normal donor (BALB/c, I-E⁺) BM cells closely resembled normal BALB/c mice in showing marked deletion of Vβ3⁺, Vβ5⁺, and Vβ11⁺ cells (but not Vβ8⁺ cells) at the level of LN CD4⁺ cells. Different results were found with TG mice not given BM cells. Except at late stages postgrafting (15 wk), these mice showed almost no deletion of Vβ11⁺ cells (relative to normal I-E⁻ BALB.B control mice). In marked contrast, deletion of Vβ3⁺ and Vβ5⁺ cells was almost as ex-

tensive as in the control I-E⁺ mice. The selective deletion of Vβ3⁺ and Vβ5⁺ cells, but not Vβ11⁺ cells, in the TG mice was seen in all mice tested and was evident in the thymus (Table 1, Exp. 2) as well as in LN.

The above data complement and extend the studies of Gollub and Palmer (12) on μ-suppressed mice, and suggest that the role of mature B cells in controlling Vβ deletion is variable, contact with B cells apparently being critical for deletion of Vβ11⁺ cells but not of Vβ3⁺ or Vβ5⁺ cells (at least for the BALB/c background). Obtaining definitive evidence on which cell types (presumably non-B cells) determine deletion of Vβ3⁺ and Vβ5⁺ cells will probably rest on defining the precise tissue distribution of the I-E-associated mouse mammary tumor virus "coligands" recognized by these T cells (13).

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