Successful Engraftment of Human Postnatal Thymus in Severe Combined Immune Deficient (SCID) Mice: Differential Engraftment of Thymic Components With Irradiation Versus Anti-asialo GM-1 Immunosuppressive Regimens

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Summary

To develop a model of human thymus growth in vivo, we have implanted postnatal human thymus under the renal capsule of severe combined immune deficient (SCID) mice and assayed for graft survival and graft characteristics 1–3 mo after engraftment. Three groups of SCID mice were engrafted with postnatal human thymus: untreated SCID mice, SCID mice pretreated with 400 cGy of γ irradiation 1–5 d before engraftment, and SCID mice treated with intraperitoneal anti-asialo GM-1 antiserum every 4–5 d during engraftment. In the untreated group of SCID mice, only 37% of grafts survived and consisted of human thymic microenvironment components and human immature thymocytes. Irradiation of SCID mice before engraftment improved survival of human thymic grafts to 83%, but these grafts were largely devoid of thymocytes and contained only thymic microenvironment components with large numbers of thymic macrophages. Treatment of SCID mice with anti-asialo GM-1 antiserum throughout the engraftment period also promoted human thymus engraftment (70%) and induced SCID B cell Ig production (SCID[Ig^+]) in 38% of animals. In SCID(Ig^-) anti-asialo GM-1–treated mice, the human thymic grafts were similar in content to those in untreated SCID mice. However, in anti-asialo GM-1–treated animals with grafts that became SCID(Ig^+), all animals were found to have mouse-human chimeric grafts in that the human thymic microenvironment (human fibroblasts, thymic epithelium, vessels) was colonized by murine T cells. These data demonstrate that human postnatal thymus will grow as xenografts in SCID mice, and that the components of human thymus that engraft are dependent on the immunosuppressive regimen used in recipient mice. A striking finding in this study was the induction of T and B lymphopoiesis in SCID mice by abrogation of NK cell activity with in vivo anti-asialo GM-1 treatment. These data strongly suggest that asialo $GM\text{-}1^+$ NK cells and/or macrophages play a role in mediation of suppression of lymphopoiesis in SCID mice.

Recent studies have begun to clarify the early stages of human T cell maturation (1–5). However, a major problem remains in the study of the human T cell lineage in that the intact thymic microenvironment is necessary for T cell precursors to mature through all stages of normal T cell development. The essential requirement of the microenvironment in T cell ontogeny necessitates that the components of thymic stroma be present in models of T lineage development. The use of animal models of T cell development have provided powerful systems to unravel complex cellular interactions that occur in thymic development (6, 7). What is needed in the study of human T cell maturation is a model system that allows for analysis of thymocyte-thymic microenvironment interactions that occur in vivo.

In 1988, McCune et al. (8) described a system in which human fetal thymic and fetal liver grafts grew in C.B-17 scid/scid (SCID) mice. In the SCID-hu model of McCune et al. (8), a reduction in the growth potential of thymic xenografts was observed when donor human thymic tissue was >20 wk of gestational age. Unlike human fetal tissue that is not routinely available to most investigators, postnatal human thymic tissue is available as discarded tissue, removed to expose the heart in select pediatric corrective cardiovascular surgical procedures.

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In this study, we have studied the growth potential of explants of human postnatal thymus in SCID mice. We demonstrate that human postnatal thymus will grow as xenografts in SCID mice, and that the components of human thymus engrafted vary with the immunosuppressive regimen given to the SCID mouse recipient. Whereas grafts in untreated animals contained both human microenvironment and thymocyte components, grafts in animals irradiated with 400 cGy before engraftment contained predominantly thymic microenvironment components only. When SCID mice were treated with anti-asialo GM-1, an antiserum against murine NK cells and macrophages, 38% of animals were induced to produce murine Ig (i.e., became SCID[Ig^+] [9]). In this group of SCID(Ig^+) mice, human thymic grafts grew well, were highly lymphoid, but, in contrast to untreated and irradiated SCID recipients, contained human thymic microenvironment components that were populated by murine T cells.

Materials and Methods

Animals. Breeder pairs of SCID (10) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) from the colony of L. Shultz, and were bred and maintained under pathogen-free conditions in the animal barrier facility at the Duke University Vivarium. Mice were housed in microisolator cages, and all food, caging, and bedding were autoclaved before use. Mice received daily trimethoprim/sulfamethoxazole in suspension in drinking water (40 mg trimethoprim and 200 mg sulfamethoxazole per 320 ml of drinking water). All mice used were between 8 and 19 wk of age.

Placement of Human Thymic Xenografts in SCID Mice. Human postnatal thymic tissue (age, 1 d to 14 mo) was obtained from patients undergoing corrective cardiovascular surgical procedures that require removal of thymic tissue for heart exposure. Thymic tissue was either used immediately for implantation into SCID mice or frozen in RPMI 1640 + 7.5% dimethyl sulfoxide + 15% FCS, and stored in liquid nitrogen for future use.

For engraftment of human thymic tissue into SCID mice, male and female SCID mice were anesthetized with a 1:1 mixture of Ketamine, 5 mg/ml (Aveco, Fort Dodge, IA) and Xylazine, 5 mg/ml, (Mobay, Shawnee, KA) at the dosage of 0.05 mg/gm. A 2-cm right flank incision was made and the kidney exteriorized. Human postnatal thymic tissue was cut into 1-mm^3 pieces and placed under the renal capsule using fine forceps. 5-0 sutures were used to approximate the peritoneal layers, and michel clips were placed to close the wound. All surgical procedures were performed under sterile conditions in a laminar flow hood.

After 1–3 mo, mouse thymus, spleen, liver, and kidney with human xenograft were removed, snap frozen in a dry ice/ethanol slurry, and stored in liquid nitrogen. Mouse peripheral blood (PB)[1] was processed for surface staining of mononuclear cells using anti-human and anti-mouse mAb reagents and analyzed on a FACS Star Plus (Becton Dickinson & Co., Mountain View, CA).

Immunosuppressive Treatment of SCID Mice. Animals engrafted with human postnatal thymic tissue received either no treatment, pretreatment (1–5 d before engraftment) with sublethal whole body γ irradiation (400 cGy) (11), or received long-term intraperitoneal administration (every 4–5 d after graft placement) of 25 μl of anti-asialo GM-1 (Wako, Richmond, VA), a rabbit antiserum against murine NK cells and macrophages (12, 13). Using indirect immunofluorescence (IF) assays on human thymus tissue or human thymocytes in suspension, we found anti-asialo GM-1 serum did not react with human thymocytes. Moreover, NK activity in SCID mice pretreated with the IFN inducer, polyinosinic-polycytidylic acid (poly I/poly C) (as measured by ^{51}Cr release assay against YAC-1 target cells), was reduced by 92% 4 d after a single injection of anti-asialo GM-1 as compared with control SCID mice injected with normal rabbit serum.

Antibodies. The following anti-human mAbs were used: CD1a (NA1/34; from A. McMichael, Oxford University) (14), CD3 (Leu4; from R. Evans, Roswell Park Memorial Institute, Buffalo, NY) (15), CD4 (T4/19, Thy-5D7; from E. Reinherz, Harvard University), CD8 (OKT8; American Type Culture Collection, Rockville, MD), CD7 (3A1a and 3A1e) (16), CD45 (F10-89-4) (17) and CD45RA (F8-11-13) from R. Dalchau and J. Fabre, Oxford University) (18), CD45RO (UCHL-1; from P. Beverley, University College, London) (19), fibronectin (FN-15; Sigma Chemical Co., St. Louis, MO), CD44 (A1G3) (20), CD29 (VLAβ, K20; from A. Bernard, Nice, France) (21), anti-HLA-A2 (MAS 2.1; from M. McCune, Systemix, Inc., Palo Alto, CA) (8), anti-HLA class I monomorphic (3F10) (22), anti-HLA class II DR monomorphic (L243) (23), anti-HLA antibodies against polymorphic HLA determinants 88.2 (DR5), DR7M (DR7), JS-1 (DR3), GS359-13F10 (DR4), 295-3E5 (DQ3) 4AA7 (DQ1+4), 296-8F9 (DQ monomorphic), 8.1 (B8), 295-8D8 (B5), 145.2 (B27), 20.1 (A2, Aw69) and 192.1 (A9, A32 B4) (all from S. Radka, Genetic Systems, Seattle, WA), V2 (anti-human vessels), A2B5 (anti-GQ ganglioside/ antimedullary thymic epithelium [TE]) (24), TE4 (antimedullary TE) (25), TE7 (anti-human fibroblasts) (25), TE3 (anti-human cortical TE and fibroblast) (26), TE8 (anti-human Hassall's bodies) (27), TCR-δ1 (anti-TCR-δ, M. Brenner, Harvard University) (28), βF1 (anti-TCR-β; M. Brenner) (29), AE-3 (anti-human keratin; from H. Sun, NYU, New York) and 12/1-2 (anti-HTLV-1 p19) reactive with subcapsular cortical and medullary TE cells (30).

Antibodies against mouse molecules included anti-TCR-β (H57-597; R. Kubo, National Jewish Hospital, Denver, CO) (31), CD4 (L3T4; Becton Dickinson & Co.), CD8 (YTS169.4; Sera Labs, Sussex, UK), Thy-1 (NIMR-1; Sera Labs), mouse macrophages (M1/70.15; Sera Labs), mouse NK cells (SW5E6; V. Kumar, Southwestern Medical Center, Dallas, TX) (32), and mouse CD3ϵ (145-2C11; J. Bluestone, University of Chicago, Chicago, IL) (33). Each antibody was used at an optimal saturating concentration as determined from titration experiments. Control antibodies included either irrelevant mouse mAbs, P3 × 63/Ag8 ascites, or directly labeled murine myeloma paraproteins.

Immunohistologic Staining of Mouse Tissues. Tissue specimens were embedded in OCT compound (Lab Tek Products, Naperville, IL), cut into 4-μm sections, and fixed in acetone (−40°C) for 5 min. Hematoxylin (H) and eosin (E) stains were performed on tissue sections of engrafted kidneys every 120 μm. Tissue sections were used immediately for immunohistologic staining or were stored at −70°C.

Anti-human mouse mAbs were either directly fluoresceinated or used in indirect IF assay with FITC goat anti-mouse IgG (GAM-FITC) (Kirkegaard & Perry Laboratories, Inc., Gathersburg, MD). Anti-mouse mAbs were either directly fluoresceinated or used in indirect IF assays with mouse anti-rat Ig FITC, (OX12-FITC; Sera Labs). Direct and indirect IF was performed on acetone-fixed, 4-μm tissue sections as previously described (34). For determination of the relative proportions of human immune cells present in thymic xenografts, the number of CD7, CD3, CD4, CD8, CD1, TCR-β,

[1] *Abbreviations used in this paper:* DP, double positive; E, eosin; GAM, goat anti-mouse; H, hematoxylin; Hh-1, hematopoietic histocompatibility 1; hpf, high-powered field; IF, immunofluorescence; PB, peripheral blood; poly I/polyC, polyinosinic-polycytidylic acid; SP, single positive; TE, thymic epithelium.

(βF1), and TCR-δ (TCR-δ1) -positive cells were visually counted using indirect IF and expressed as an estimated percent (to nearest 5%) of human CD45$^+$ cells present (35). For determination of the relative proportions of murine immune cells present in human thymic xenografts and in mouse lymphoid organs, the number of TCR-β, CD3, CD4, and CD8 -positive cells were visually counted using indirect IF and expressed as an estimated percent (to nearest 10%) of murine Thy-1$^+$ cells present (35).

ELISA for Quantitation of SCID Mouse Ig Levels. A two-site ELISA was used to determine serum levels of murine IgM and IgG. Briefly, goat anti-mouse F(ab')$_2$ fragments (anti-F[ab']$_2$ fragment specific) were coated onto high-bind EIA microtiter wells (Costar, Cambridge, MA) (2 h, 25°C). The plates were blocked with 3% BSA (1 h, 25°C) and washed three times with 0.05% Tween 20 in PBS. Twofold dilutions of SCID mouse sera samples were incubated in microtiter wells (1 h, 25°C). After washing, alkaline phosphatase-conjugated anti-mouse IgM (μ specific) (Fisher Scientific Co., Pittsburgh, PA) or anti-mouse IgG (γ specific) (Fisher Scientific Co.) was added (1:1,000 dilution) (1 h, 25°C). The amount of mouse IgM or IgG bound was measured by hydrolysis of paranitrophenyl phosphate (Sigma Chemical Co.) at 405 nm in an Anthros 2001 ELISA reader (Denley Instruments, Durham, NC). IgM and IgG antibody concentrations for SCID mice were calculated by linear regression analysis from a standard curve generated using purified myeloma proteins of known concentrations: TEPC 183 (IgM, κ) (Sigma Chemical Co.) or MOPC 195 (IgG$_{2b}$, κ) (Litton Bionetics, Kensington, MD). Mice were determined to be "leaky" (SCID[Ig$^+$]) if murine serum IgM and IgG levels were >50 μg/ml (9, 36). Alternatively, in preliminary studies, we also found that reaction of GAM-FITC with SCID mouse tissue was an indicator of SCID(Ig$^+$) status, and the reactivity of GAM-FITC with mouse kidney was used to indicate SCID(Ig$^+$) status in select animals in whom serum was not available.

Results

Engraftment of Human Postnatal Thymus in SCID Mice. Table 1 shows the effect of irradiation and anti-asialo GM-1 treatment on the rate of engraftment of human postnatal thymus in SCID mice. Of 84 total mice operated, 47 viable grafts were present after 1–3 mo (56%). 43 untreated SCID animals had human thymic tissue implanted, and after 1–3 mo, 16 (37%) had viable thymus grafts present. In contrast, pretreatment of SCID mice with either sublethal whole body γ irradiation (400 cGy) or treatment with anti-asialo GM-1 improved human thymic graft survival to 83 and 70%, respectively.

Table 1. *Human Postnatal Thymic Xenograft Survival in SCID Mice after No Treatment, Treatment with 400 cGy Whole body γ Irradiation, or Anti-asialo GM-1 Treatment*

Treatment	No. operated	No. engrafted*	Percent engraftment
No treatment	43	16	73%
400 cGy gamma irradiation	18	15	83%
Anti-asialo GM-1	23	16	70%
Total	84	47	56%

* Engraftment of human postnatal thymic tissue was defined as visible human thymic tissue present at the time of autopsy that was confirmed as human in origin by immunohistologic staining using anti-human mAbs.

The average leukocyte counts in engrafted untreated SCID mice were similar to leukocyte counts previously reported in nongrafted SCID mice (37) (Table 2). Both sublethal irradiation of SCID mice and treatment with anti-asialo GM-1 significantly lowered the lymphocyte count at the time of graft harvest ($p < 0.005$ and 0.02, respectively). However, only treatment with anti-asialo GM-1 lowered the PB monocyte count ($p < 0.001$). When PB mononuclear cells were analyzed for a subset of NK cells as defined by mAb SW5E6 (33), we found that both irradiation and anti-asialo GM-1

Table 2. *PBL Counts in SCID Mice Engrafted with Human Thymus after Various Treatment Regimens*

Treatment	No. of mice tested	Leukocytes	Neutrophils	Lymphocytes	Monocytes	SW5E6$^+$ NK cells*
		mm^3	mm^3	mm^3	mm^3	mm^3
No treatment	46	2,576 ± 258	1,643 ± 186	853 ± 83	79 ± 17	167 ± 41
400 cGy γ irradiation	15	2,014 ± 400	1,685 ± 325	419 ± 118‡	146 ± 70§	28 ± 15‖
Anti-asialo GM-1	24	2,074 ± 441	2,120 ± 370	567 ± 96¶	16 ± 6**	34 ± 20‡

At the time of autopsy (1–3 mo after implantation of human thymus tissue), mice were bled retroorbitally. Differential counts were performed on Wright-stained PB smears, and total leukocyte counts were determined on a cell counter (Coulter Immunology). Results are expressed as mean number of PB cells per mm^3 ± SEM.

* Indirect IF and flow cytometry analysis using NK cell marker SW5E6 was also performed on a subset of mice within each treatment group. PB was taken 4–5 d after injection with anti-asialo GM-1 antisera. For number of SW5E6$^+$ NK cells, $n = 9$ for untreated group, $n = 8$ for irradiation group, and $n = 11$ for anti-asialo GM-1 group.

‡ Significantly different from untreated group ($p < 0.005$).

§ Not significantly different from untreated group ($p < 0.7$).

‖ Significantly different from untreated group ($p < 0.01$).

¶ Significantly different from untreated group ($p < 0.02$).

** Significantly different from untreated group ($p < 0.001$).

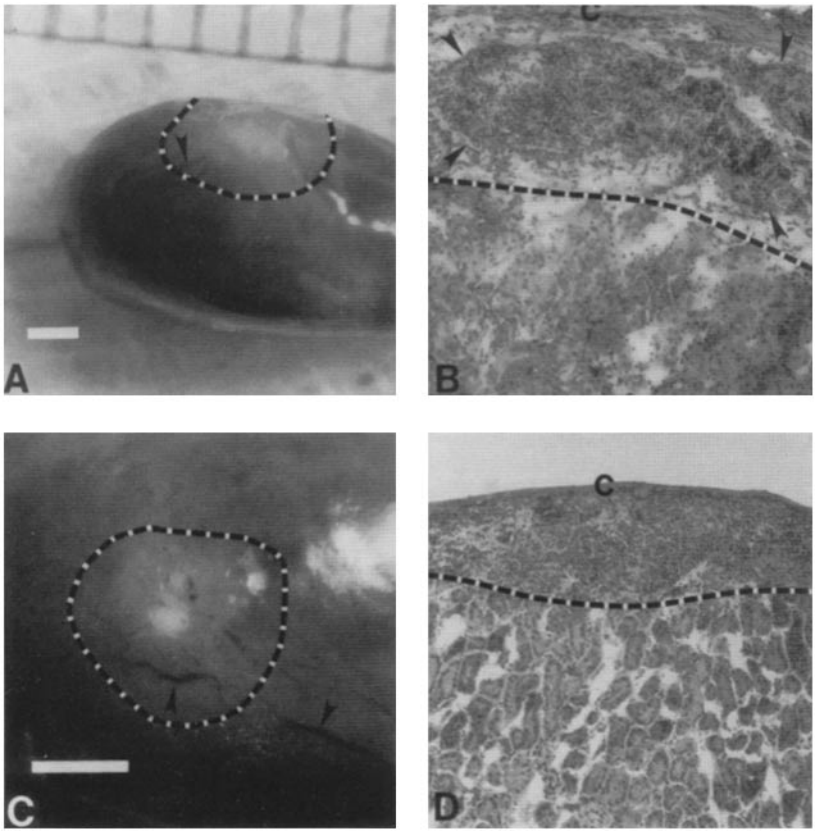

Figure 1. Gross and histologic morphology of human postnatal thymic grafts implanted under the renal capsule of SCID mice. (*A*) A macroscopic view of thymic xenograft 11-6 (3 × 2 mm), 6 wk after placement under the renal capsule. (*B*) Low-power view of graft 11-6 under the renal capsule (*c*). The human thymic graft is outlined by black arrows. The dotted line indicates the graft-kidney interface. (*C*) A thymic xenograft (2 × 2 mm), 10 wk after placement under the renal capsule of SCID mouse 14-2. (*D*) Low-power view of graft 14-2 documents the histology of the graft underneath the renal capsule (*c*). Dotted line points out graft-kidney interface. Solid white bar in *A* and *C* = 1 mm (*B* and *D*, ×100; H and E stain).

treatment significantly decreased the number of SCID PB SW5E6$^+$ NK cells by 83 and 80%, respectively.

We next determined if human leukocytes could be detected in mouse PB at the time of autopsy after 1–2 mo of engraftment. Using two-color flow cytometry with directly conjugated CD7-FITC and CD45-PE reagents, rare CD45$^+$, CD7$^-$ cells (69 ± 7 cells/mm^3) were present in PB in engrafted SCID mice in the untreated, engrafted group (n = 3). No circulating human CD45$^+$ cells could be found in engrafted SCID mice treated with either irradiation (n = 13) or anti–asialo GM-1 (n = 12). Immunohistologic analysis of SCID mouse spleen and mediastinal thymus from untreated animals engrafted with human thymus showed no detectable CD7$^+$ or CD3$^+$ human cells in these mouse organs (data not shown).

All human thymus tissue used as xenografts were screened in indirect IF assays on tissue sections for reactivity with monomorphic and polymorphic HLA class I and class II mAbs before and following engraftment. Monomorphic and polymorphic HLA class II mAbs reacted with only human TE

Table 3. *Characterization of Human Postnatal Thymic Grafts Placed in Untreated SCID Mice*

Graft No.	Days after engraftment	Thymic epithelium			Fibroblast	Vessels	Macrophages	Human thymocytes							
		Keratin	TE4	TE3	(TE7)	(V2)	(Leu-M3)	CD45	CD7	CD3	CD4	CD8	CD1	TCR-β	TCR-δ
2-2	30	+	+	+	+	+	−	100	100	100	80	100	100	ND	0
2-5	46	+	−	+	+	+	−	100	30	20	15	Rare	0	Rare	Rare
3-1	45	+	+	+	+	+	−	100	100	100	100	100	0	100	Rare
6-3	51	+	+	+	+	ND	ND	Rare	0	0	0	0	0	0	0
6-9	55	+	−	+	+	+	−	100	10	ND	30	0	0	15	0
3-8	57	+	+	+	+	+	−	100	100	100	80	80	Rare	100	Rare

Numbers represent estimated percent of cells positive relative to the number of CD45$^+$ cells, with the number of CD45 cells at 100%. Rare, one to two cells positive per slide. +, cells present reactive with mAb in IF assays. −, no cells present reactive with mAb in IF assays. Similar data were obtained in an additional five grafts in untreated SCID mice (data not shown).

cells and macrophages in thymic grafts while HLA monomorphic and polymorphic HLA class I mAbs reacted with human TE cells, fibroblasts, and vessels. In 18 grafts tested among all treatment groups, polymorphic HLA antigens expressed by the thymic graft were identical to the polymorphic HLA antigens expressed by the thymic tissue before engraftment (data not shown).

Fig. 1 shows the gross and histologic morphology of representative mouse kidneys (nos. 11-6 and 14-2) bearing 6- and 10-wk human thymic grafts. After 1–2 mo, the human grafts routinely increased in size by threefold. However, when the human thymic grafts from each treatment regimen were tested for the presence of human thymic microenvironment components and for thymocyte subsets, we found the xenografts in each treatment group were dramatically different.

Characterization of Human Thymic Grafts in Untreated SCID Mice. After 1–2 mo of engraftment, grafts in the untreated SCID mouse group contained both human thymic microenvironment components and varying numbers of human thymocytes (Table 3). Immunohistologic analysis of representative graft 2-2 (Fig. 2) shows the presence of human thymic microenvironment components, including human fibroblasts and keratin-positive thymic epithelium as defined by anti-human fibroblast marker, TE7 (Fig. 2 *B*), anti-human keratin antibody, AE-3 (Fig. 2 *C*), and by the medullary TE cell marker, TE4 (Fig. 2 *D*). Of 10 grafts studied using immunohistologic techniques, all had TE7$^+$ thymic fibroblasts present. Moreover, in 9 of 10 grafts, TE7$^+$ cells invaded the renal parenchyma to a depth of 100–400 μM (Fig. 2 *B*). 3 of 10 grafts in untreated mice had only TE7$^+$ fibroblasts present with

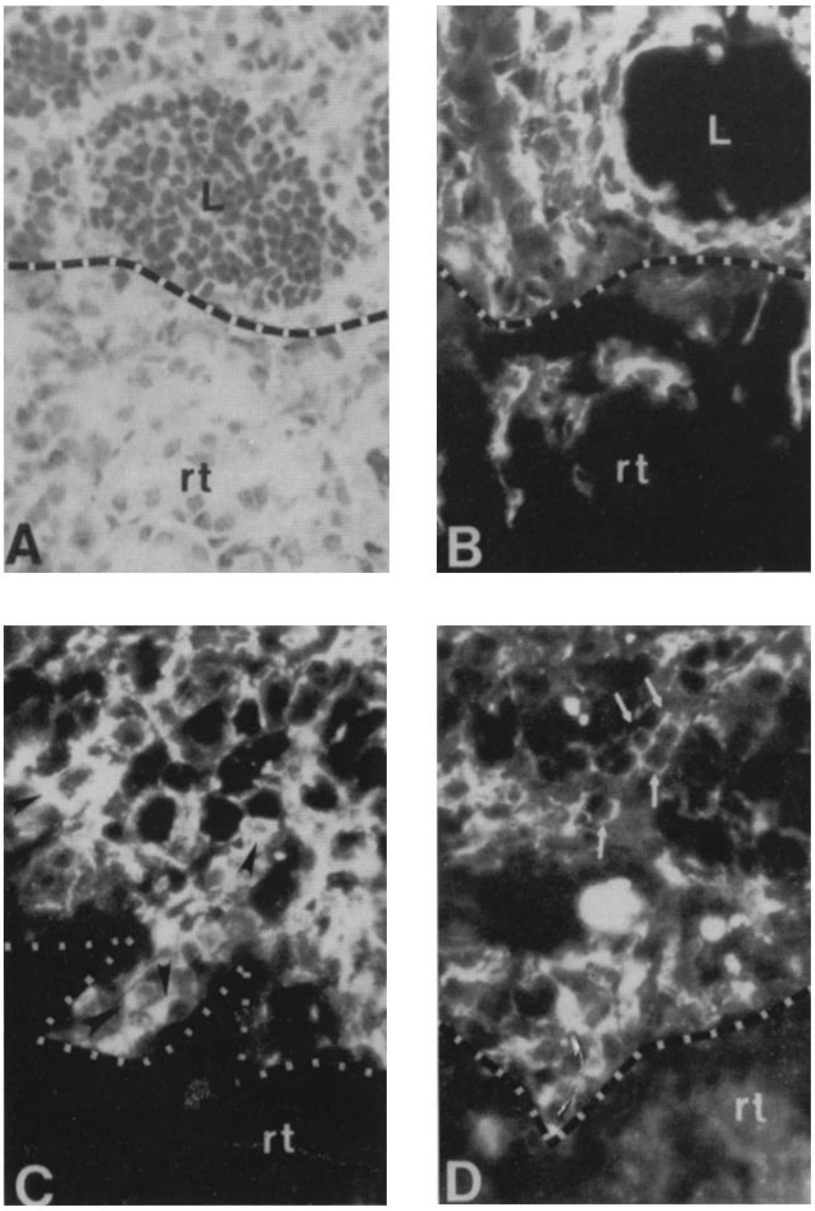

Figure 2. Phenotypic analysis using indirect IF assays of a human postnatal thymic xenograft, 4 wk after implantation under the renal capsule of SCID mouse 2-2. (*A*) H and E stain of graft no. 2-2; *L* indicates lymphoid area, dotted line outlines border of graft, *rt* points out renal tubules. (*B*) TE7$^+$ thymic fibroblasts surrounding a TE7$^-$ lymphoid area (*L*); TE7$^+$ cells are seen invading the renal parenchyma and surrounding renal tubules (*rt*). (*C*) Keratin-positive thymic epithelium using anti-human keratin antibody AE-3; arrows point to AE-3$^+$ TE cells. (*D*) Medullary thymic epithelium as identified by TE4, an antimedullary TE cell marker (×400).

no AE-3/TE4$^+$ TE cells present, while the remaining seven grafts in this group also contained keratin-positive TE cells.

As seen in Figs. 1, *B* and *D*, and 2 *A*, human postnatal thymic grafts in SCID mice lost their normal morphology and assumed a dysplastic morphology, with only rare Hassall's bodies present. None of the thymic grafts studied using indirect IF assay had a definable cortico-medullary junction. In grafts that contained TE cells, TE cells formed islands of keratin-positive, TE4$^+$, A2B5$^+$, p19$^+$ thymic epithelium, suggesting that the predominant epithelial cell type was medullary and subcapsular cortical thymic epithelium (24–26, 30). In all but two grafts tested, the TE4$^+$ medullary epithelium was also TE3$^+$ (Fig. 3). In normal thymus, TE4$^+$ medullary and subcapsular cortical thymic epithelium is mutually exclusive of TE3$^+$ cortical epithelium, except for a minor subset of TE3$^+$, TE4$^+$, normal TE cells in the subcapsular cortical area (26). TE3 is also normally expressed intracellularly in thymic fibroblasts and macrophages (26, 38). Thus, in postnatal thymus grafts in SCID mice, thymic epithelium was not normal and expressed both TE4 and TE3, a phenotype identical to that seen only in a rare subset of normal subcapsular cortical TE cells (26).

Human vessels were present throughout thymic grafts in the untreated SCID mouse group, and varied in size from small capillaries to large muscular arteries. As with TE7$^+$ cells, V2$^+$ endothelial cells routinely invaded the underlying renal parenchyma. In two to five grafts tested, human vessels were positive for human fibronectin, LFA-3, ICAM-1, and VLA-β (data not shown) (reviewed in references 1, 39, and 40).

In untreated SCID mice with human thymic grafts, the human lymphoid component of the thymic graft varied in number and in phenotype. Table 3 shows an analysis of six human grafts present in untreated SCID mice. Human lymphoid cells present in untreated SCID mice were either CD4$^+$, CD8$^+$, CD1$^+$ cells (graft 2-2) (Fig. 4), CD4$^+$, CD8$^+$, CD1$^-$ cells (grafts 3-1 and 3-8), or of less mature phenotypes (grafts 6-3 and 6-9). When reacted with either βF1 (anti-TCR-β) and TCR-δ1 (anti-TCR-δ), most thymocytes were TCR-β^+ (grafts 3-1 and 3-8) with only rare (one to two cells positive per slide) TCR-δ^+ cells present. In grafts 6-3 and 6-9, most of the CD45$^+$ cells present were both TCR-β^- and TCR-δ^- (Table 3). In three grafts (9-4, 9-5, and 9-13), only TE7$^+$ fibroblasts were present without either CD45$^+$ human lymphoid cells or keratin-positive TE cells (data not shown).

Fig. 4 shows the lymphoid component of a 4-wk human postnatal thymic graft (no. 2-2) in an untreated, engrafted SCID mouse. Fig. 4, *B–F*, shows human thymocytes in the graft stained with human T cell lineage markers CD7, CD4, CD8, CD3, and CD1; most thymocytes present in graft 2-2 reacted with all of these antibodies. Interestingly, in untreated SCID mice, using antibody Leu-M3 as a human macrophage marker, we found only rare Leu-M3$^+$ cells (Table 3). Using mAbs specific for mouse T cells (CD3ϵ, Thy-1), we found that 50% of grafts in this group contained rare (1-3 cells/section) murine T cells. Finally, of the untreated mice with viable human thymic grafts, none were found to be SCID (Ig$^+$) at the time of graft harvest.

Characterization of Human Postnatal Thymus Grafts in SCID Mice Pretreated with 400 cGy γ Irradiation. In contrast to untreated SCID mice, when animals were treated with 400 cGy γ irradiation before engraftment, the resulting grafts were comprised primarily of thymic microenvironment components only, and were markedly depleted of human or murine T cells in seven of nine grafts tested. Table 4 summarizes the characterization of nine thymus grafts studied from animals that had been irradiated before engraftment. In contrast to human thymus grafts in untreated SCID mice, Leu-M3$^+$ macrophages were observed in irradiated SCID mice in seven of nine thymus grafts and were present in large numbers (>20

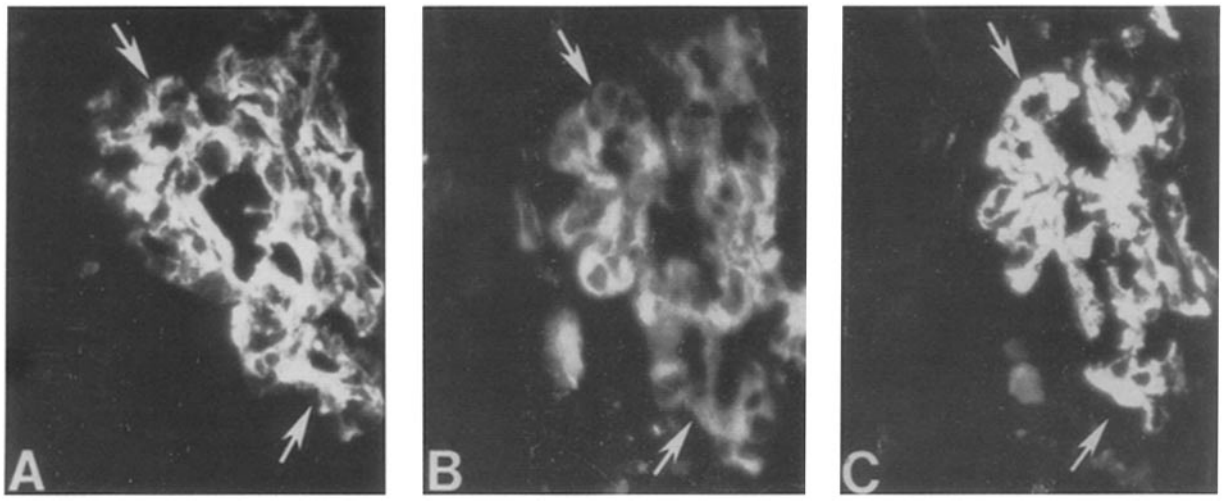

Figure 3. Phenotypic analysis using indirect IF assays of human thymic epithelium in a human xenograft placed under the renal capsule of SCID mouse 3-8. Figure shows sequential 4-μm frozen sections of the same island of thymic epithelium in the thymic xenograft from SCID mouse 3-8. (*A*) TE cells are keratin positive, as determined by reactivity with mAb AE-3. Moreover, the same TE cells also are TE3$^+$ (*B*), and TE4$^+$ (*C*) (×400).

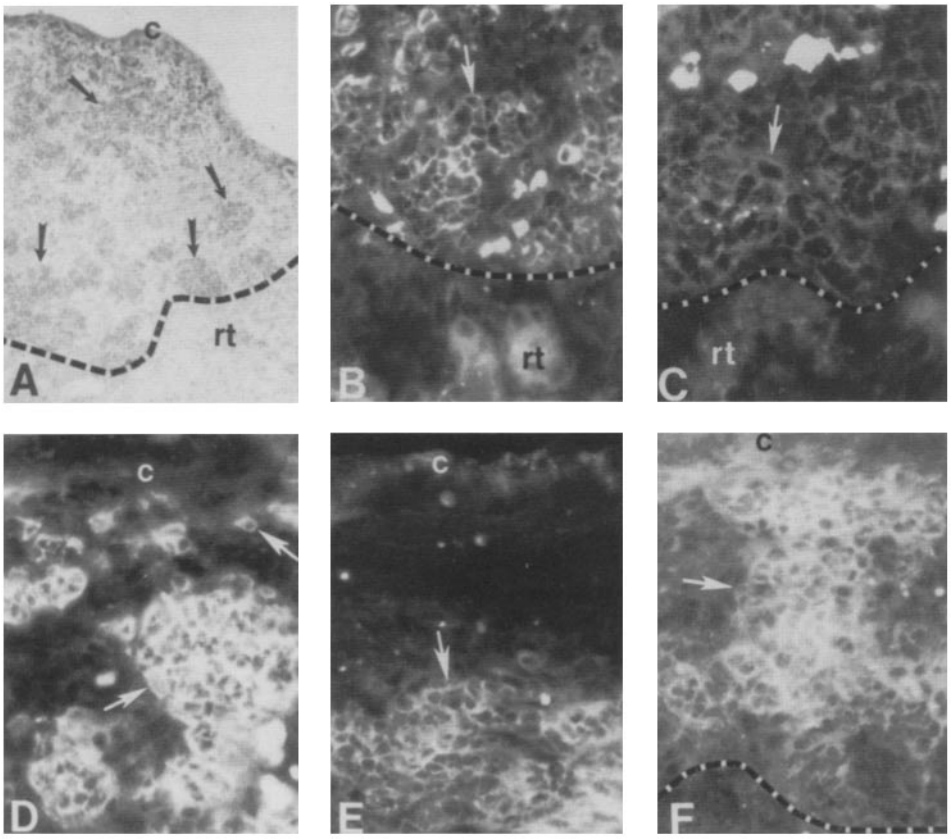

Figure 4. Phenotypic analysis using indirect IF assays of a human postnatal thymic xenograft, 4 wk after implantation under the renal capsule of SCID mouse 2-2. (*A*) H and E stain of graft 2.2; arrows point out thymocyte aggregates, dotted line delineates border of graft, *c* indicates renal capsule, *rt* indicates renal tubules. Phenotypic analysis of human thymocytes as defined by CD7 (*B*), CD4 (*C*), CD8 (*D*), CD3 (*E*), and CD1 (*F*). Dark area under renal capsule in *E* is space between the graft and the capsule and not $CD3^-$ human T cells (*B*, *C*, *D*, *E*, and *F*, ×400; *A*, ×100).

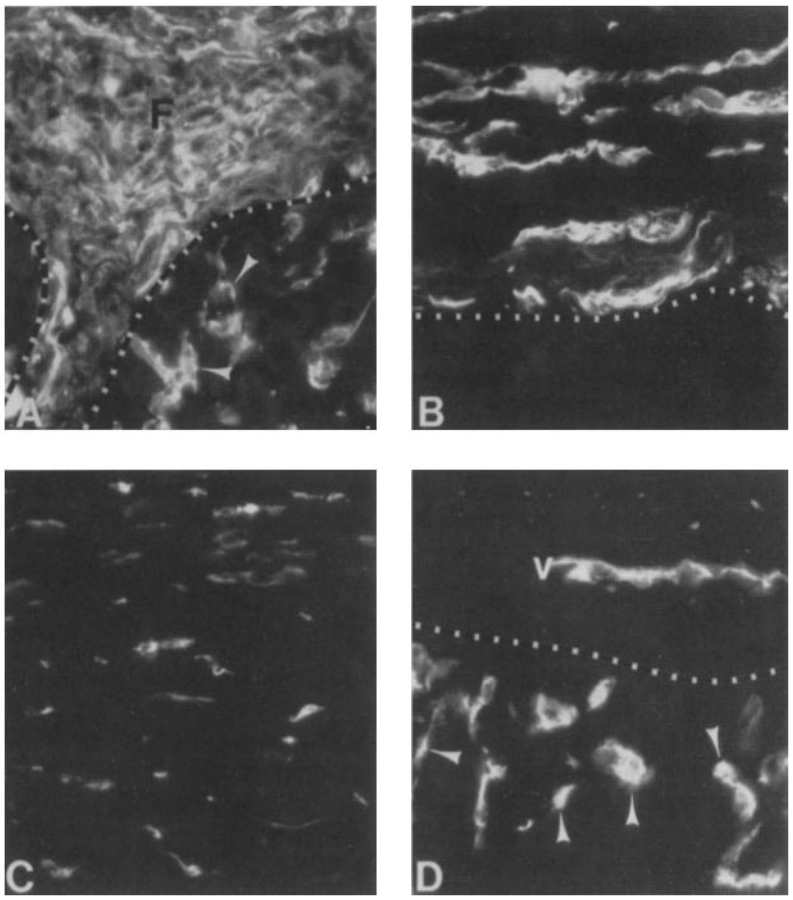

Figure 5. Phenotypic characterization using indirect IF assays of human thymic graft in SCID mouse 13-1 irradiated with 400 cGy before engraftment. (*A*) Human fibroblasts present, reactive with antibody TE3. Fibroblasts are seen invading the mouse kidney below the graft-kidney interface (*dotted line*). (*B*) Keratin-positive human thymic epithelium, as identified by mAb AE-3, is present in graft (area above dotted line). (*C*) $CD45^+$ human thymic macrophages, also found to be Leu-M3 and Mo-1^+, were present throughout the graft. (*D*) V2 reactivity with human vessels (*v*) and endothelial cells (*arrows*). $V2^+$ endothelial cells invaded the renal parenchyma beyond the graft-kidney interface (*dotted line*) (×400).

cells/high-powered field [hpf]) in five grafts. Fig. 5 shows a representative human thymus graft (no. 13-1) in the irradiation treatment group. Graft 13-1 contained human fibroblasts (Fig. 5 *A*), keratin-positive human thymic epithelium (Fig. 5 *B*), numerous human thymic macrophages (Fig. 5 *C*), and V2$^+$ endothelial cells (Fig. 5 *D*). Human V2$^+$ endothelial cells routinely invaded the murine renal parenchyma (Fig. 5 *D*), as did TE7$^+$ human fibroblasts (data not shown). Only two of nine animals studied in this group had clusters of CD45$^+$, CD3$^+$ human T cells present in thymus grafts. Most of the T cells present in graft 8-11 were CD4$^-$, CD8$^-$, TCR-β^+ cells, while CD1$^+$, CD4$^+$, CD8$^+$, TCR-β^+ cells predominated in graft 8-14 (Table 4). Otherwise, only thymic microenvironment components predominated in human thymic grafts in irradiated animals. As with the untreated SCID group, either no or rare murine T cells were present in human thymic grafts placed in irradiated SCID recipients. In addition, no irradiated mice with viable human xenografts were found to be SCID(Ig$^+$) at the time of graft harvest.

Characterization of Human Postnatal Thymus Grafts in SCID Mice Treated With Anti-asialo GM-1. To further facilitate engraftment of human thymic tissue, we treated SCID animals with anti-asialo GM-1 antiserum to decrease NK cell and monocyte/macrophage number and function (12, 13). Of 23 grafts implanted in this group, 16 (70%) human thymic grafts survived and grew. Surprisingly, at 1–2 mo after engraftment, of 16 animals with viable thymus grafts, six (38%) had become SCID(Ig$^+$).

In anti-asialo GM-1–treated animals that were SCID(Ig$^-$) at autopsy, thymus grafts were similar to grafts in untreated

Table 4. *Characterization of Human Postnatal Thymic Grafts Placed in SCID Mice Pretreated with 400 cGy γ Irradiation*

Graft No.	Days After engraftment	Thymic epithelium			Fibroblast (TE7)	Vessels (V2)	Macrophages (Leu-M3)	Human thymocytes							
		Keratin	TE4	TE3				CD45	CD7	CD3	CD4	CD8	CD1	TCR-β	TCR-δ
13-1	30	+	+	+	+	+	+	Rare	Rare	Rare	NA	Rare	LC	Rare	0
13-2	30	+	+	+	+	+	+	Rare	Rare	Rare	Rare	Rare	LC	Rare	0
13-4	30	+	+	+	+	+	+	Rare	Rare	Rare	NA	Rare	0	Rare	0
13-5	30	+	+	+	+	+	+	Rare	Rare	Rare	Rare	Rare	0	Rare	0
8-5	30	+	+	+	+	+	+	Rare	0	Rare	NA	0	0	0	0
8-6	30	+	+	−	+	+	−	Rare	0	0	NA	0	0	0	0
8-11	30	+	+	+	+	+	+	100	Rare	Rare	NA	Rare	Rare	100	0
8-12	30	+	+	+	+	−	−	0	0	0	0	0	0	0	0
8-14	30	+	+	+	+	+	Rare	100	80	50	80	80	80	50	0

Numbers represent estimated percent of cells positive relative to the number of CD45$^+$ cells present, with the number of CD45 cells at 100%. Rare, 5–10 cells/slide. +, cells present reactive with mAb in IF assay. −, no cells present reactive with mAb in IF assay. LC, rare CD1$^+$ dendritic cell present consistent wih Langerhan's cells. NA, not applicable since macrophages were CD4$^+$ and therefore rare thymocytes could not be evaluated.

Table 5. *Characterization of Human Postnatal Thymic Grafts in Anti-asialo GM-1 Antiserum-treated SCID (Ig$^-$) Mice*

Graft No.	Days after engraftment	Thymic epithelium			Fibroblast (TE7)	Vessels (V2)	Macrophages (Leu-M3)	Human thymocytes							
		Keratin	TE4	TE3				CD45	CD7	CD3	CD4	CD8	CD1	TCR-β	TCR-δ
11-2	30	+	+	+	+	+	Rare	100	0	15	15	50	0	30	0
11-3	30	−	−	−	+	+	−	0	0	0	0	0	0	0	0
11-4	30	+	−	+	+	+	−	100	0	30	0	0	0	0	0
11-5	30	+	+	+	+	+	−	100	10	100	10	0	50	50	20
11-6	43	+	+	+	+	+	Rare	100	100	100	100	40	20	100	0
11-7	43	+	+	+	+	+	Rare	Rare	Rare	Rare	NA	0	0	Rare	0
14-7	89	+	+*	+	+	+	Rare	100	50	100	50	0	0	50	0

Numbers represent estimated percent of cells positive relative to the number of CD45$^+$ cells, with the number of CD45 cells at 100%. Rare, 5–10 cells/slide. +, cells present reactive with mAb in IF assay. −, no cells present reactive with mAb in IF assay. NA, not applicable since macrophages were CD4$^+$ and therefore rare thymocytes could not be evaluated.

* For 11-7, TE cells were p19$^+$, another marker of medullary TE cells (29).

Table 6. *Characterization of Human Postnatal Thymic Grafts in Anti-asialo GM-1-treated SCID (Ig⁺) Mice*

Graft No.	Days after engraftment	Thymic epithelium			Fibroblast (TE7)	Vessels (V2)	Macrophages (Leu-M3)	Human thymocytes (CD45)	Murine thymocytes				
		Keratin	TE4	TE3					CD3	TCR-β	CD4	CD8	Thy-1
10-6	65	+	+	ND	+	ND	−	0	100	100	100	Rare	100
10-7	82	+	+	ND	+	+	−	Rare	100	100	60	40	100
11-9	71	−	−	−	+	ND	−	0	Rare	Rare	0	0	Rare
11-10	71	+	ND	ND	+	−	−	0	100	10	20	0	100
14-2	89	+	+	ND	+	−	−	0	100	100	80	20	100
14-9	89	+	+	ND	+	+	−	0	100	100	80	20	100

For murine T cells, numbers represent the estimated percent of cells positive relative to the number of Thy-1⁺ cells, with the number of Thy-1⁺ cells at 100%. Rare, 5–10 cells/slide. +, cells present reactive with mAb in IF assay. −, no cells present reactive with mAb in IF assay.

animals, with thymic microenvironment components with varying numbers of thymocytes, and only rare Leu-M3⁺ macrophages present (Table 5).

In five of six anti-asialo GM-1-treated mice that were SCID(Ig⁺) at the time of graft harvest, large lymphoid grafts were present under the renal capsule (Fig. 1, *C* and *D*). However, in striking contrast to anti-asialo GM-1-treated SCID(Ig⁻) mice, none of the lymphoid cells in human thymic grafts in SCID(Ig⁺) anti-asialo GM-1-treated mice were human T cells. Rather, 100% of the lymphoid cells colonizing human thymic microenvironment components were murine CD3ε⁺, TCR-β⁺ SCID T cells (Table 6, Fig. 6). In direct IF assays, the chimeric thymus grafts contained murine T cells that varied in phenotype from all CD4⁺ single-positive (SP) cells (no. 10-6) to mixtures of CD4⁺ and CD8⁺ cells (nos. 10-7, 14-2, and 14-9) (Table 6). In double staining experiments on grafts from animals 14-2 and 14-9, the expression of murine CD4 and CD8 was found

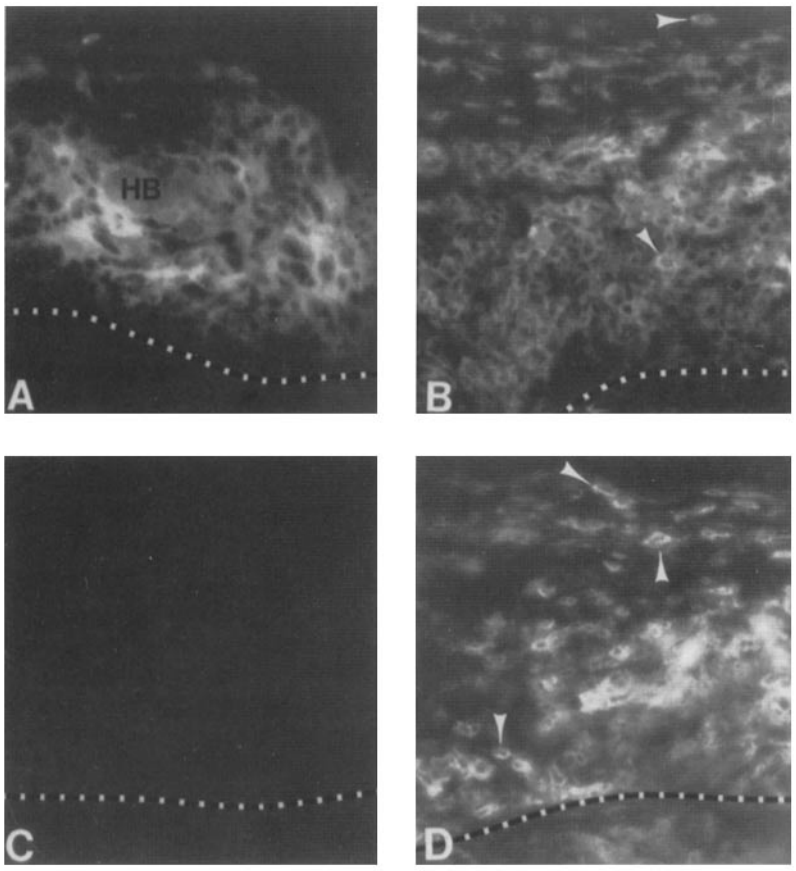

Figure 6. Phenotypic characterization using direct IF assays of human-mouse chimeric thymic graft in anti-asialo GM-1-treated SCID(Ig⁺) mouse 14-2 after engraftment with human postnatal thymus. (*A*) HLA A2⁺ human thymic epithelium, as defined by reactivity with mAb MA2.1, (anti-HLA A2). A central human Hassall's body (*HB*) was present. (*B*) All lymphoid cells present in graft 14-2 were murine CD3ε⁺ using mAb 145-2C11, while (*C*) none of the lymphoid cells present in graft 14-2 were reactive with the anti-human CD45 antibody, F-10-89-4. (*D*) The majority the murine CD3ε⁺ lymphoid cells present in the graft 14-2 were TCR-β⁺, using the anti-murine TCR-β mAb, H57-597. Dotted line in all panels indicates the graft-kidney interface (×400).

to be mutually exclusive in the majority of mouse lymphocytes (~90%), although these techniques could not rule out the presence of a small proportion of $CD4^+$, $CD8^+$ double-positive (DP) cells. Murine TCR-β^+ T cells were present in and around human thymic epithelium, and as well were scattered throughout $TE7^+$ human thymic fibroblasts. $TE7^+$ cells invaded the renal parenchyma, 100–400 μM, and murine T cells were scattered in interstitial areas of the mouse kidney in a similar zone around the human graft. In the kidney interstitium, mouse T cells were frequently in close proximity to human $TE7^+$ cells (data not shown).

Determination of the Effects of Anti-asialo GM-1 Treatment on Induction of SCID(Ig^+) Status in SCID Mice. In our initial set of experiments, we found that 38% of anti-asialo GM-1–treated mice with thymus xenografts became SCID(Ig^+) 2–3 mo after engraftment, as compared with 0% of mice in the untreated and irradiated groups. To clarify the factors responsible for induction of SCID(Ig^+) status, age-matched SCID(Ig^+) mice were treated with either anti-asialo GM-1 alone, anti-asialo GM-1 plus engraftment, or normal rabbit serum (50 μl, i.p.), and the presence of serum mouse Ig was determined after 60 d. Of nine SCID(Ig^-) mice treated with normal rabbit serum every 4 d, none became SCID(Ig^+) after 2 mo. However, in 10 SCID(Ig^-) mice treated with anti-asialo GM-1 every 4 d but not engrafted, four (40%) became SCID(Ig^+) after 2 mo. Moreover, of an additional 35 anti-asialo GM-1–treated, engrafted animals, 14 (40%) became SCID(Ig^+) after 2 mo. Taken together, these data demonstrate that long-term intraperitoneal administration of anti-asialo GM-1 antiserum alone led to induction of SCID (Ig^+) status in ~40% of SCID mice, suggesting a role for NK cell– and/or monocyte-mediated suppression of lymphopoiesis in these animals.

Phenotype of Murine T Lineage Cells in SCID Mouse Lymphoid Tissues in Various Groups of SCID Mice. We next evaluated mouse lymphoid cells present in mouse spleen and thymus of SCID mice in all three treatment groups (Table 7). First, as normal controls, three age-matched (161 d), untreated, nonengrafted SCID mice were screened for mature mouse T cells in spleen and thymus. As reported previously, SCID (Ig^-) mouse thymus contained Thy-1^+, $cCD3^+$, TCR-β^- cells with rare $CD4^+$, $CD8^+$ cells present (42). In one age-matched SCID(Ig^+) animal (no. 26-2), rare splenic TCR-β^+ cells were detected. Similarly, untreated SCID mice (134–177 d) that were engrafted for 30–57 d contained only rare $CD4^+$ or $CD8^+$ cells in spleen and thymus. Anti-asialo GM-1–treated and engrafted mice (113–175 d; engrafted for 30–89 d) that remained SCID(Ig^-) at the time of autopsy had clusters of $CD4^+$ and $CD8^+$ thymocytes present in two of three animals, with rare $CD4^+$ or $CD8^+$ cells present as well in the spleen. In contrast, anti-asialo GM-1–treated mice (154–162 d; engrafted for 71–89 d) that became SCID(Ig^+)

Table 7. *Phenotypic Analysis of Murine T Lineage Cells in Mouse Lymphoid Tissues from SCID Mice in Various Treatment Groups*

Mouse type	SCID Mouse	Mouse thymus				Mouse spleen			
		CD3	CD4	CD8	TCR-β	CD3	CD4	CD8	TCR-β
Nonengrafted, untreated SCID mice*									
	26-1	80	Rare	Rare	0	–	Rare	Rare	–
	26-2‡	80	Rare	Rare	0	Rare	Rare	Rare	Rare‡
	26-3	80	Rare	Rare	0	–	Rare	Rare	–
Engrafted, untreated SCID mice									
	2-2	90	Rare	0	0	Rare	Rare	Rare	–
	3-8	80	Rare	0	Rare	–	+	–	–
	6-9	ND	Rare	0	Rare	–	+	–	–
Engrafted, anti-asialo GM-1–treated, SCID(Ig^-) mice									
	11-2	90	80	90	Rare	–	Rare	Rare	–
	11-6	80	0	0	0	–	–	–	–
	14-7	80	80	80	Rare	–	+	Rare	–
Engrafted, anti-asialo GM-1–treated, SCID(Ig^+) mice									
	14-2	100	50	50	10	+	+	+	+
	14-9	80	80	80	10	+	+	+	+
	11-10	100	50	90	0	+	+	+	–

Phenotypic analysis performed as in Table 6.
Rare is one to five positive cells per three to five in hpf. +, large clusters of positive cells (>10 cells/hpf) present around splenic arterioles. –, no cells positive.
* In nongrafted control SCID mice, no human thymic grafts were implanted.
‡ 26-2 was SCID(Ig^+) at time of study.

had large clusters of murine TCR-β^+ cells in mediastinal thymus and in spleen in two of three animals tested (nos. 14-2 and 14-9). Moreover, large numbers (10–50 cells/hpf) of $CD4^+$ and/or $CD8^+$ cells in thymus and spleen were present in all three anti-asialo GM-1-treated animals that became SCID(Ig^+). One such animal (no. 11-10) had large numbers of $CD4^+$ and/or $CD8^+$ cells in thymus and spleen, but no TCR-β^+ cells present. Interestingly, this animal also had subsets of murine $cCD3^+$, $CD4^-$, $CD8^-$, TCR-β^- and $cCD3^+$, $CD4^+$, $CD8^-$, TCR-β^- cells in the human thymus graft, documenting in this animal the homing of immature SCID mouse T cells to the human thymic xenograft (Table 6).

Discussion

In this study, we have shown that human postnatal thymic tissue will grow as xenografts in SCID mice. The components of the human thymic grafts at 1–3 mo varied with the immunosuppressive regimen used in the SCID mouse recipient. Xenografts from untreated SCID mice contained both human thymic microenvironment and thymocytes, whereas irradiation of SCID mice before engraftment yielded primarily human thymic microenvironment grafts containing human macrophages but devoid of human or murine thymocytes. Treatment of SCID mice with anti-asialo GM-1 after engraftment resulted in murine B cell antibody production (SCID[Ig^+]) in 38% of animals. Remarkably, in five of the six SCID(Ig^+) mice, the human thymic grafts grew well, were highly lymphoid, but in contrast to untreated and irradiated SCID recipients, contained exclusively murine TCR-β^+ T cells.

Mice homozygous for the SCID mutation exhibit markedly impaired lymphopoiesis, while myelopoiesis is unaffected (10, 43). Despite this selective deficiency, SCID mice have normal levels of NK cells and NK cell precursors and show levels of NK cell activity similar to their C.B-17 counterparts (44–46). While in vivo treatment with anti-asialo GM-1 antiserum has been used in several mouse strains to abrogate NK activity, its use in SCID mice to enhance engraftment of human tissue has not been reported.

The observation that anti-asialo GM-1 treatment alone induced ~40% of SCID mice to become SCID(Ig^+) was a striking finding in our study and may provide insights into cellular controls of the recombinase defect in SCID mice (47, 48). Induction of both T and B cell lymphopoiesis by anti-asialo GM-1 antiserum raises the notion that there may be a cell-mediated suppression of B and T cell lymphopoiesis involving NK cells, macrophages, or both cell types in SCID mice. For example, SCID NK cells and/or monocytes could act by eliminating or suppressing the function of cells responsible for the production of cytokines necessary for induction of recombinase in SCID T and B cell precursors (reviewed in reference 49). Alternatively, SCID NK cells could suppress SCID lymphopoiesis by selective NK-mediated killing of autologous SCID lymphoid precursors. Murphy et al. (50) have recently demonstrated in the setting of bone marrow transplantation in SCID mice that NK cells can inhibit lymphopoiesis, in that NK cells can mediate the rejection of T cell–depleted bone marrow allografts. Murphy et al. (51) have gone on to show that adoptively transferred SCID NK cells can mediate hematopoietic histocompatibility 1 (Hh-1)-mediated bone marrow rejection in vivo. Taken together with the data in our current study, these observations raise the hypothesis that SCID NK cells may mediate killing of autologous SCID lymphocyte precursors via the Hh-1 antigen system.

In those anti-asialo GM-1–treated SCID(Ig^+) animals in which human thymus grafts were present, the grafts were chimeric in that they contained human thymic microenvironment components colonized exclusively by murine TCR-β^+ T cells. The homing of murine TCR-β^+ T cells to the human thymic microenvironment in SCID mice treated with anti-asialo GM-1 could represent an attempt at thymus graft rejection, or alternatively, could represent maturation of SCID mouse T cell precursors in the human thymic microenvironment. In mouse 11-10, the murine T cells were TCR-β^- and were immature, demonstrating homing of immature mouse T cells to the human xenograft at least in this one animal. If the presence of murine T cells in human thymic microenvironment grafts proves to represent colonization of human thymic stroma with developing mouse thymocytes, then this model system should be important for the determination of optimal methods for reconstitution of the human T cell lineage across HLA barriers. Murine CTL precursors have been reported to be positively selected during development to recognize human HLA-A2 as a restricting element in HLA-A2 transgenic mice (52). In nude mice engrafted with xenogenic rat thymus, chimeric grafts develop with mouse T cell precursors colonizing the rat thymic microenvironment (53). Moreover, we have recently shown human TE cells can bind to SCID mouse thymocytes in vitro (Singer, K.H., and B.F. Haynes, unpublished results). Thus, it is plausible that developing mouse T cells could colonize a human thymic microenvironment.

Human thymocytes were rare in grafts in anti-asialo GM-1–treated SCID(Ig^+) mice and in irradiated SCID mice. In irradiated animals, the potential is present for vascular injury to occur due to irradiation, predisposing thymocytes to die out due to delayed vascularization. Moreover, although we showed human thymocytes and thymic macrophages were anti-asialo GM-1 negative in indirect IF assays, anti-asialo GM-1 could react with human thymocytes or with macrophages at levels too low to detect in vitro in IF assays, yet react at levels sufficient to result in thymocyte loss in vivo.

It is not clear why human/mouse chimeric grafts were seen in anti-asialo GM-1–treated mice and not seen in irradiated SCID mice. Total body sublethal γ irradiation has been shown to markedly reduce murine NK activity (54). 400 cGy of γ irradiation has been reported to be required for engraftment of SCID mice with syngeneic bone marrow (9); this level of γ irradiation has also been shown to damage SCID B lymphocyte precursors (11). Thus, it is likely that 400 cGy irradiation damaged murine lymphoid precursors and prevented murine T and B cell lymphopoiesis both in SCID hemato-

poietic microenvironments and in the engrafted human thymic microenvironment.

The presence of numerous Leu-M3$^+$ tissue macrophages in thymic microenvironment grafts in irradiated SCID recipients is intriguing and suggests that macrophage precursors are present in human thymus that are capable of continuously growing in irradiated but not in untreated or anti-asialo GM-1–treated SCID mice (55, 56). In the Mosier et al. (57) SCID mouse model, human monocytes did not engraft well, while in the McCune et al. (8) model, engrafted human fetal liver contained precursors of thymic dendritic cells that did engraft.

In the present study, in all three groups of SCID mice, there was a hierarchy of cell survival in human thymic grafts placed, with fibroblasts and endothelial cells surviving longer than TE cells, and TE cells surviving longer than thymocytes. The paucity of thymocytes in several of the human thymic grafts in untreated SCID mice could indicate a limited regenerating capacity of postnatal T cell precursor cells within human thymus. In mice, reconstitution of bone marrow chimeras with murine postnatal CD4$^-$,CD8$^-$,CD3$^-$ thymocytes only transiently reconstituted mouse mature thymocyte populations (58, 59). While we observed human thymic grafts containing TE cells with no thymocytes, we did not see human thymic grafts with thymocytes in the absence of human TE cells. These data suggest dependence of thymocytes on TE cells, and dependence of TE cells on fibroblasts and endothelial cells to grow in vivo (reviewed in references 1 and 38). Indeed, a role for fibroblast-produced cytokines on human TE cell growth has been suggested to be important in early thymic organogenesis (reviewed in reference 38).

Finally, it is important to compare the system described in the present report with the SCID-hu system of McCune et al. (8). The simultaneous transplantation of human fetal thymus and fetal liver by McCune et al. gave rise to thymic grafts containing the thymic microenvironment from the human donor thymus and thymocytes and dendritic cells from the fetal liver (8). Experiments were not reported on the fate of human fetal thymus grafts placed in SCID mice alone, although thymus grafts from donors >20 wk of gestational age were reported to grow less well than grafts from earlier tissues (8). Our study demonstrates that human postnatal thymus tissue from children of 1–14 mo gestational age grow in SCID mice and routinely increased in size by threefold over a 60-d period. The size increase observed in our study was less than that reported by McCune et al., perhaps for two reasons: (*a*) none of our engrafted animals were simultaneously given a source of bone marrow or fetal liver stem cells; and (*b*) all tissue was postnatal, possibly with reduced growth potential as compared with fetal thymus tissue. Against the latter possibility is the observation that in children who undergo multiple thoracotomies for corrective cardiovascular surgery in whom >95% of thymic tissue was removed at the initial operation, the thymus is frequently observed to have regenerated by the time of subsequent operations (R. Ungerleider, Duke University, personal communication). Presently, experiments are underway to attempt to reconstitute human thymic microenvironment grafts in irradiated SCID mice with human bone marrow CD7$^+$ T cell precursors (1, 2) to resolve this issue. Namikawa et al. (60) have recently reported long-term survival of multilineage hematopoietic precursors in fetal thymus grafts implanted with fetal liver grafts. In contrast to the thymic grafts described by Namikawa et al. (60) and McCune et al. (8), our thymic grafts have a dysplastic morphology (Fig. 3). If reconstitution experiments using T cell precursors in our system does not promote normal postnatal thymic growth and histogenesis, then this could be a major limitation of the SCID-Hu model using postnatal thymus.

Thus, the use of human postnatal thymic tissue grafts in immunosuppressed SCID mice provides an easily accessible model for the study of human thymocyte development and human thymic microenvironment function. Moreover, study of the mechanisms of action of anti-asialo GM-1 in inducing T and B cell lymphopoiesis should provide insights into the cellular control of lymphopoiesis. Finally, study of human/mouse chimeric thymus grafts in SCID(Ig$^+$) anti-asialo GM-1–treated mice provides a unique model to study evolutionarily conserved mechanisms of TE-thymocyte interactions.

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