

TRANSPLANTATION OF THYMUS TISSUE IN COMPLETE DIGEORGE SYNDROME

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ABSTRACT

Background The DiGeorge syndrome is a congenital disorder that affects the heart, parathyroid glands, and thymus. In complete DiGeorge syndrome, patients have severely reduced T-cell function.

Methods We treated five infants (age, one to four months) with complete DiGeorge syndrome by transplantation of cultured postnatal thymus tissue. Follow-up evaluations included immune phenotyping and proliferative studies of peripheral-blood mononuclear cells plus biopsy of the thymus allograft. Thymic production of new T cells was assessed in peripheral blood by tests for T-cell–receptor recombination excision circles, which are formed from excised DNA during the rearrangement of T-cell–receptor genes.

Results After the transplantation of thymus tissue, T-cell proliferative responses to mitogens developed in four of the five patients. Two of the patients survived with restoration of immune function; three patients died from infection or abnormalities unrelated to transplantation. Biopsies of grafted thymus in the surviving patients showed normal morphologic features and active T-cell production. In three patients, donor T cells could be detected about four weeks after transplantation, although there was no evidence of graft-versus-host disease on biopsy or at autopsy. In one patient, the T-cell development within the graft was demonstrated to accompany the appearance of recently developed T cells in the periphery and coincided with the onset of normal T-cell function. In one patient, there was evidence of thymus function and CD45RA+CD62L+ T cells more than five years after transplantation.

Conclusions In some infants with profound immunodeficiency and complete DiGeorge syndrome, the transplantation of thymus tissue can restore normal immune function. Early thymus transplantation — before the development of infectious complications — may promote successful immune reconstitution. (N Engl J Med 1999;341:1180-9.)

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THE DiGeorge syndrome is a congenital malformation that affects the development of the third and fourth pharyngeal pouches and is associated with a deficiency of T cells.¹⁻³ Many patients with the DiGeorge syndrome are hemizygous for 22q11^{4,5}; in rare instances, patients are hemizygous for 10p13.⁶ Deletion in the *UFDIL* gene at 22q11 has been described.⁷ The term complete DiGeorge syndrome is used to de-

scribe patients with the syndrome who have profound T-cell deficiency.⁸⁻¹²

Several therapies have been used to treat immunodeficiency associated with the DiGeorge syndrome. In two patients, HLA-identical bone marrow transplantation successfully restored T-cell function by adoptive transfer of mature T cells.^{13,14} Immune reconstitution was reported after transplantation of peripheral-blood mononuclear cells (PBMCs) in one patient.¹⁵ In a few cases, transplantation of fetal thymus was followed by immune reconstitution.¹⁶⁻¹⁹ However, some of those patients had partial DiGeorge syndrome with detectable T-cell function before transplantation and might have improved without therapy.^{11,19} Most published trials of postnatal thymus transplantation have been unsuccessful.²⁰⁻²² Transplantation of bone marrow stem cells has not been successful.¹²

We report our experience with a series of five infants with complete DiGeorge syndrome who were treated with allogeneic, cultured, postnatal thymus tissue. We hypothesized that host T cells would develop in the donor thymus allograft and would lead to reconstitution of immune function. In the last patient in our series (and in the first patient, with the use of cryopreserved samples), we also tested the hypothesis that markers of newly formed naive T cells would appear only after thymus transplantation and would correlate with the development of T-cell proliferative function.

METHODS**Thymus Transplantation**

We obtained discarded thymus tissue from infants 2 to 35 days old who were undergoing corrective heart surgery after we received informed consent from the donors' parents. Down's syn-

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drome was a criterion for exclusion of donors. Preparation of the thymus tissue for transplantation has been described elsewhere.²³ In Patient 5, a Stadie-Riggs hand microtome (Thomas Scientific, Swedesboro, N.J.) was used to slice the thymus tissue.²⁴ The slices of thymus tissue were inserted into the quadriceps bilaterally in an open procedure in the operating room.^{23,25}

Immune Testing and Determination of the T-Cell-Receptor Repertoire

We evaluated immune function through standard procedures,²⁶ including flow cytometry and measurement of the incorporation of [³H]thymidine after stimulation of PBMCs (cultures of 10⁵ cells) with phytohemagglutinin and concanavalin A. For studies of flow cytometry, we used murine monoclonal antibodies to CD3, CD4, CD8, CD19, CD20, and CD16 (AMAC, Westbrook, Me.; Coulter, Hialeah, Fla.; Dako, Carpinteria, Calif.; and Becton Dickinson, Mountain View, Calif.). We performed HLA typing using serologic²⁷ and molecular²⁸ techniques. Additional murine monoclonal antibodies were used to characterize the T-cell receptor V_β (TCRBV) repertoire in Patients 3 and 5 (Coulter; Endogen, Woburn, Mass.; and PharMingen, San Diego, Calif.).

Immunoperoxidase Staining of Frozen and Formalin-Fixed Sections

Four-micrometer frozen sections underwent reaction with antibodies using an avidin-biotin-peroxidase complex technique (Vector Laboratories, Burlingame, Calif.).²³ Antibodies directed against thymic-epithelium (TE) antigens included cytokeratin; TE3, expressed on cortical epithelium; TE4, on subcapsular cortical and medullary epithelium; and TE16, on Hassall's corpuscles (clusters of terminally differentiated thymic epithelial cells, which are characteristic of normal thymic medulla). Additional antibodies used in this study on paraffin-embedded tissue included KP-1 (CD-68), which is reactive with macrophages, and S-100, which is reactive with dendritic cells (both from Dako).

Quantification of the Output of the Thymus

We quantified the output of the thymus by measuring the excisional DNA products of T-cell-receptor gene rearrangements (T-cell-receptor recombination excision circles [TRECs]),²⁹ which exist as episomes in T cells and can be detected by the polymerase chain reaction. We performed the assay for signal-joint TRECs²⁹ on PBMCs separated into CD4+ and CD8+ cells, using magnetic microbeads (MACS, Milteny Biotec, Auburn, Calif.). The median numbers of TRECs in four cord-blood samples were 9584 per 100,000 CD4+ cells (range, 9257 to 16,948) and 9729 per 100,000 CD8+ cells (range, 8244 to 14,998). The median values in two normal infants, each studied three times between birth and six months of age, were 10,360 per 100,000 CD4+ cells (range, 7520 to 10,722) and 10,663 per 100,000 CD8+ cells (range, 8026 to 10,997). In nine normal children between 1.9 and 7.4 years of age, the median numbers of TRECs were 11,170 per 100,000 CD4+ cells (range, 7800 to 14,473) and 8413 per 100,000 CD8+ cells (range, 5887 to 13,961).

RESULTS

Patient 1

Patient 1, whose initial course after transplantation on day 90 of life has been reported previously,^{23,30} presented with no circulating T cells and no T-cell proliferative responses to mitogens (this patient was described as Patient 4 in a previous study¹²) (Table 1). Within the first month after transplantation of a cultured postnatal thymus allograft from an unmatched male donor into this infant girl, an oligoclonal population of T cells developed that did

not proliferate in response to stimulation with mitogens.^{23,30} On day 17 after transplantation, these CD3+ T cells (2024 per cubic millimeter) were shown to be female by fluorescence in situ hybridization of 101 sorted T cells. Thus, these T cells were not derived from the allograft.

An evaluation of an allograft biopsy at three months with the use of HLA monoclonal antibodies revealed host thymopoiesis (T-cell development in the thymus) within donor-thymus epithelium.^{23,30} In the patient, robust T-cell proliferative responses to mitogens developed,²³ and a normal TCRBV repertoire³⁰ developed approximately seven months after transplantation of the thymus. In mixed-lymphocyte reaction, the patient's T cells were unresponsive to the donor's PBMCs.²³ B-cell antibody responses to immunization with tetanus toxoid and pneumococcal vaccine were normal.²³ Five years later (two years after the initial report),²³ the patient had normal responses to mitogens, CD3 stimulation, and mixed-lymphocyte culture (Table 2), had normal proliferative and antibody responses to tetanus toxoid, and was a normal child.

Patient 2

Patient 2 was born with multiple anomalies of CHARGE association (coloboma, heart disease, atresia choanae, retarded growth and development, genital hypoplasia, and ear abnormalities, deafness, or both) (this patient was previously described as Patient 5¹² and as Patient 1³³).³⁴⁻³⁶ This boy had profound immunodeficiency with no T cells detectable by flow cytometry and no proliferative responses to mitogens in PBMCs before transplantation on day 96 of life. On day 49 after the patient underwent transplantation with a cultured postnatal thymus allograft from a haploidentical female donor, the peripheral-blood T-cell proliferative response to phytohemagglutinin was more than 200,000 counts per minute (cpm). This response fell by day 64 after transplantation, probably as a result of steroid therapy that was given after an intracranial hemorrhage. Because of the patient's unexpected death on day 66 after transplantation, there was no opportunity to immunize the patient and assess antigen-specific responses. Phytohemagglutinin-stimulated PBMCs obtained on day 64 after transplantation, two days before the baby's death, were analyzed by fluorescence in situ hybridization for X chromosome markers. The analysis showed 15 percent XY (host) and 85 percent XX (thymus-donor) cells. A polymerase-chain-reaction test for HLA-DR that was performed with an aliquot of these cells also revealed the presence of donor HLA-DR alleles. Thus, this patient had evidence of large numbers of engrafted donor T cells.

An autopsy was performed. No evidence of graft-versus-host disease was found. No native thymus tissue was found despite careful gross and microscopi-

TABLE 1. CLINICAL FEATURES OF FIVE PATIENTS WITH THE DIGORGE SYNDROME WHO UNDERWENT THYMUS-TISSUE TRANSPLANTATION.*

PATIENT No.	SEX	AGE AT TRANSPLANTATION (DAYS)	22q111	PRESENTATION		PROCEDURES	CLINICAL COURSE	OUTCOME
				CARDIAC FEATURES	NONCARDIAC FEATURES			
1	F	90	Normal	PDA	GER, hypocalcemic seizure	Nissen fundoplication, gastrostomy, central-line placement	One episode of sepsis requiring ventilation for 1 wk	Alive and well at home 5.75 yr after transplantation (6 yr of age)
2	M	96	Normal	PDA	Tracheomalacia, GER, ear anomaly, hypocalcemia, coloboma, microphallus, cleft lip and palate	PDA ligation, tracheostomy, cleft-lip repair, central-line placement, Nissen fundoplication, gastrostomy	<i>Staphylococcus epidermidis</i> sepsis, intracranial hemorrhage	Death from brain hemorrhage on day 66 after transplantation (162 days of age)
3	M	51	Hemizygous	Aortic narrowing, connection of left subclavian vein to coronary sinus, patent foramen ovale, dilated coronary sinus	Small omphalocele, malrotation, malformed ear	Gastrostomy, tracheostomy, central-line placement, liver biopsy	Superior vena cava syndrome, hepatomegaly, cholangiolar cholestasis, total parenteral nutrition, respiratory failure requiring mechanical ventilation for 3 mo, hypothyroidism	Death from sepsis and respiratory failure on day 130 after transplantation (181 days of age)
4	F	127	Hemizygous	ASD, PDA, right aortic arch	Cleft soft palate, abnormalities of left finger and left foot, hypocalcemia, severe GER, tracheomalacia	Central-line placement, Nissen fundoplication, gastrostomy-tube placement, laparotomy	<i>S. epidermidis</i> bacteremia, vancomycin-resistant enterococcus sepsis, thrombocytopenia, hypothyroidism, CMV infection, continuous ventilation for final 4 mo of life	Death from sepsis and respiratory failure 45 days after transplantation (5.7 mo of age)
5	M	63	Normal	Dilated coronary sinus, mild peripheral pulmonary stenosis, small PDA	Laryngomalacia, bilateral colobomas, nasopharyngeal reflux, abnormal peristalsis of hypopharynx, cricopharyngeal achalasia, micrognathia, glossoptosis, high-arched palate, microphallus, profound hearing loss, transient hypocalcemia, failure of ossification of hyoid bone [†]	Gastrostomy-tube placement	No major infections, hearing aids	Alive and well at home 11 mo after transplantation (13 mo of age); receiving IVIG and trimethoprim-sulfamethoxazole; weekly speech, occupational, and physical therapy; fed by gastrostomy

*PDA denotes patent ductus arteriosus, GER gastroesophageal reflux, ASD atrial septal defect, CMV cytomegalovirus, and IVIG intravenous immune globulin.

†The 10p13 site was not examined because of the rarity of its deletion.

TABLE 2. IMMUNOLOGIC EVALUATION OF PATIENT 1, 5.5 YEARS AFTER THYMIC TRANSPLANTATION.*

TEST	PATIENT	ADULT CONTROL
Lymphocyte phenotype†		
CD3+		
Percent	46	65
Absolute no./mm ³	580	—
CD3+CD4+		
Percent (% CD45RA+CD62L+)	26 (41)	47 (36)
Absolute no./mm ³	328	—
CD3+CD8+		
Percent (% CD45RA+CD62L+)	13 (26)	15 (18)
Absolute no./mm ³	164	—
CD16+		
Percent	23	18
Absolute no./mm ³	290	—
CD19+		
Percent	21	6
Absolute no./mm ³	265	—
PBMC proliferation (cpm)‡		
Phytohemagglutinin	163,916	114,463
Concanavalin A	171,613	187,596
Anti-CD3, soluble	147,370	128,082
Anti-CD3, immobilized	166,793	139,550
Medium	1,351	2,784
Mixed-lymphocyte reaction (cpm)‡		
Autologous	2,125	320
Against pool	34,322	27,372

*All tests were performed 5.5 years after transplantation except the mixed-lymphocyte reaction, which was performed 4.75 years after transplantation. PBMC denotes peripheral-blood mononuclear cell, and cpm counts per minute.

†At this age (one to six years), the interquartile range (25th to 75th percentile) for the normal number of CD3+ cells is 1800 to 3000 per cubic millimeter, the normal number of CD16+ natural killer cells is 200 to 600 per cubic millimeter, and the normal number of CD19+ B cells is 700 to 1300 per cubic millimeter.³²

‡Proliferative studies were performed in triplicate in cultures of 10⁵ cells. The soluble anti-CD3 was used at 50 ng per milliliter.

cal evaluation. Cytokeratin-positive material was detected in the right and left quadriceps, showing the presence of abundant donor-thymus epithelium (data not shown). One Hassall's corpuscle was identified. No immature, cortical thymocytes were detected in the graft, possibly because of the steroid treatment that was given for 48 hours before the boy's death.

Patient 3

Patient 3 was born with multiple anomalies (Table 1), no circulating T cells, and an absence of PBMC proliferative responses to mitogens. On day 51 of life, the boy was treated with a cultured postnatal thymus allograft from a male donor matched for HLA-DR15. On day 28 after transplantation, the patient had a proliferative response to phytohemagglutinin of 139,503 cpm. Because respiratory failure developed on day 35 after transplantation, the patient was treated with high doses of steroids (40 mg per kilo-

gram of body weight of methylprednisolone sodium succinate [Solu-Medrol]) per day for three days. The number of circulating T cells decreased to 153 per cubic millimeter on day 45 after transplantation and remained below 250 per cubic millimeter in the eight samples tested subsequently. The level of proliferative responses to mitogens decreased to one to four times the base-line level (in four tests). Antigen-specific T-cell responses were not tested. The circulating T cells obtained on day 119 after transplantation were examined by fluorescence in situ hybridization. All cells were male, which ruled out maternal engraftment; they consisted of 90 percent 22q11 hemizygous cells (from the host) and 10 percent 22q11 normal cells (from the donor).

The patient died of respiratory failure on day 130 after transplantation. An autopsy revealed an absence of native thymus, thyroid, and parathyroids, a finding that confirmed the diagnosis of complete DiGeorge syndrome. Histologic evaluation revealed no evidence of graft-versus-host disease. Evaluation of the thymus allograft showed no evidence of thymopoiesis. In addition, antibodies that are usually specific for Hassall's corpuscles reacted with all epithelium (data not shown). The same pattern of reactivity was also found for antibodies that are usually specific for cortical (TE3) or medullary (TE4) epithelium. The very high dose of steroids given to this patient may have contributed to the abnormal appearance of the allograft.

Patient 4

At birth, Patient 4 was found by flow cytometry to have no CD3+ T cells and to have 576 natural killer cells per cubic millimeter and 2215 B cells per cubic millimeter. The PBMCs did not proliferate in response to mitogens. Other findings are described in Table 1. A cytomegalovirus infection developed in the patient and led to dependence on a ventilator. At 127 days of life, the girl received an unmatched thymus transplant. A small number of T cells (47 per cubic millimeter and 22 per cubic millimeter) were detected eight days and one month after transplantation, respectively. Two additional tests revealed no T cells. Because of the lack of T cells, the proliferative function of PBMCs was not assessed. The patient died of sepsis and respiratory failure 45 days after transplantation, at 5.7 months of age. Permission for autopsy was denied.

Patient 5

Patient 5 had multiple anomalies characteristic of CHARGE association (Table 1) in addition to characteristics of complete DiGeorge syndrome (Table 3). T-cell function developed steadily in this infant boy after he received postnatal cultured thymus tissue from a haploidentical female donor on day 63 of life (Table 3 and Fig. 1A). An increase in CD3+

TABLE 3. IMMUNE FUNCTION OF PATIENT 5.*

CHARACTERISTIC	AGE									
	3 DAYS	31 DAYS	44 DAYS	57 DAYS	59 DAYS	93 DAYS	133 DAYS	185 DAYS	243 DAYS	
No. of days after transplantation	-60	-32	-19	-6	-4	+30	+70	+122	+180	
Phenotype of PBMCs (cells/mm ³)†	74	197	352‡	ND	378 (0% CD45RA+ CD62L+)§	420 (3% CD45RA+ CD62L+)¶	163 (7% CD45RA+ CD62L+)¶	604 (10% CD45RA+ CD62L+)¶	1208	
CD3+CD4+		197	352	ND	338	382	143	499	1012 (65% CD45RA+ CD62L+)	
CD3+CD8+		0	0	ND	0	4	0	105	261 (58% CD45RA+ CD62L+)	
CD19+ or CD20+	428	592	1257	ND	716	ND	1142	735	1110	
CD16+	983	1877	2465	ND	497	ND	4428	708	390	
Fluorescence in situ hybridization				Cells in interphase 100% male			Cells in interphase 16% female	Proliferating T cells 100% male		
Serum IgG, IgA, and IgM				Normal						Normal**

*PBMC denotes peripheral-blood mononuclear cell. Negative numbers for days indicate days before transplantation. ND denotes not determined.

†In this age group (2 days to 11 months),³¹ the interquartile range for the normal number of CD3+ T cells is 1700 to 3600 per cubic millimeter, the normal number of B cells is 500 to 1500 per cubic millimeter, and the normal number of natural killer cells is 300 to 700 per cubic millimeter.

‡On day 44 of life, there were no detectable CD3+ T cells expressing the following T-cell-receptor V β (TCRBV) chains: TCRBV2, TCRBV5S2,S3, TCRBV14, TCRBV17, or TCRBV22.

§On day 59 of life, 1 percent of CD3+ T cells expressed each of TCRBV9, TCRBV13S3, and TCRBV17, and no cells were detectable expressing TCRBV2, TCRBV5S2,S3, TCRBV14, or TCRBV17.

¶On day 185 of life (122 days after transplantation), approximately 2 percent of CD3+ cells expressed each of TCRBV2, TCRBV5S1, TCRBV8, TCRBV9, TCRBV14, and TCRBV17, but no or very few CD3+ T cells expressed TCRBV3, TCRBV5S2, TCRBV13S3, or TCRBV22.

||The patient was receiving intravenous immune globulin.

**This sample was obtained 283 days after transplantation.

CD45RA+CD62L+ T cells, indicating cells recently formed in the thymus, was noted beginning one month after transplantation (Table 3). Computed tomography of the chest was performed with contrast three months after transplantation, when T-cell proliferative function had become robust (to deter-

mine whether the native thymus had become enlarged), but it showed no evidence of a native thymus. At four and six months after transplantation, the patient had excellent T-cell proliferative responses to allogeneic cells and tetanus toxoid (after one immunization) (data not shown). Fluorescence in situ

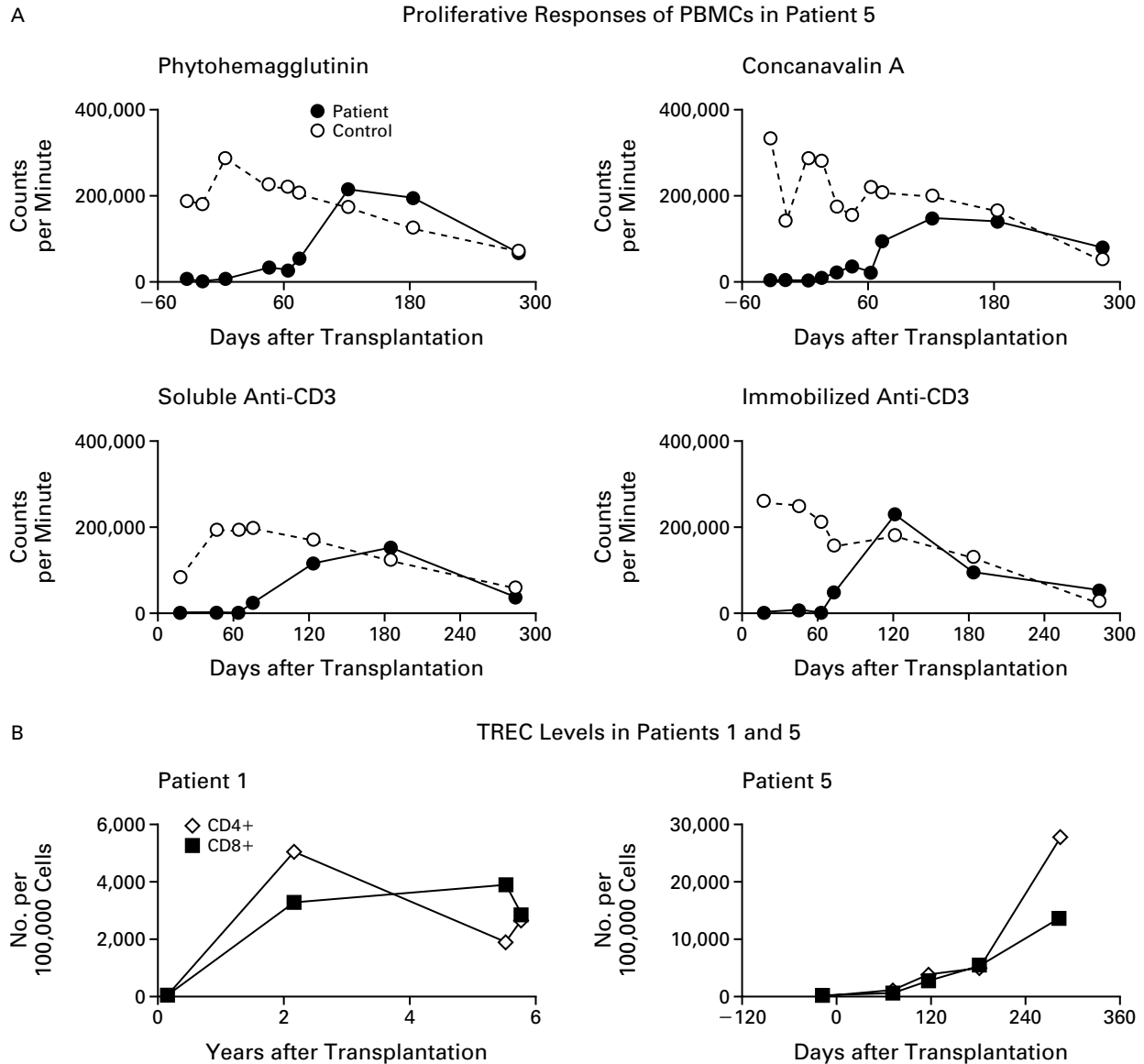


Figure 1. Proliferative Function of T Cells in Patient 5 and Levels of T-Cell–Receptor Recombination Excision Circles (TRECs) in Patients 1 and 5.

Panel A shows proliferative responses of peripheral-blood mononuclear cells (PBMCs) to phytohemagglutinin, concanavalin A, soluble anti-CD3 at 50 ng per milliliter, and immobilized anti-CD3 in Patient 5. The means of cultures of 10^5 PBMCs, in counts per minute, are shown for the patient and for an adult control. The backgrounds (medium) have been subtracted from the responses. Panel B shows data on TRECs for Patients 1 and 5. For Patient 1, there were 8700 CD3+ T cells per cubic millimeter in the blood on the date of the initial sample. Numbers of T cells were not available for the sample taken at 2.2 years. The numbers and phenotypes of T cells for the sample taken at 5.5 years are shown in Table 2. The numbers of T cells were very similar for the sample taken at 5.8 years. For Patient 5, most of the correlative data on T cells are presented in Table 3. On day 283 after transplantation, Patient 5 had 1380 CD3+ T cells per cubic millimeter, with 1050 CD3+CD4+ T cells per cubic millimeter and 300 CD4+CD8+ T cells per cubic millimeter. In both panels, negative numbers of days indicate days before transplantation, which occurred on day 0.

hybridization at four months, with the use of peripheral blood that had been sorted to include only T cells that were proliferating in response to phytohemagglutinin, showed that all T cells were male (host cells).

A biopsy of the thymus allograft performed 10 weeks after transplantation showed a clear distinction between cortex and medulla (Fig. 2A). Immunohistochemical analysis showed the cortical areas to be full of immature CD1a+ thymocytes (Fig. 2B). The Hassall's corpuscles in the medulla were normal, a finding consistent with good thymic function (Fig. 2C and 2D). In contrast to the findings in Patient 3, the TE16 antibody reacted specifically with Hassall's corpuscles (Fig. 2D), as in a normal thymus, but not with other keratin-positive epithelium (Fig. 2C). KP-1-positive cells resembling macrophages (Fig. 2E) and S-100+ dendritic cells (Fig. 2F) were present primarily in the medulla, as is found in the normal thymus.

TREC Analyses

For Patient 1, evaluations of TRECs were done on cryopreserved PBMCs from day 45 and month 26 after transplantation and on fresh samples from 5.5 and 5.8 years after transplantation (Fig. 1B). TRECs were undetectable in the initial sample but were present at approximately one third of normal levels 26 months and 5.5 and 5.8 years after transplantation. For Patient 5, TRECs were undetectable before transplantation and increased in parallel with the increase in the numbers of CD45RA+CD62L+ T cells and in T-cell function (Table 3 and Fig. 1).

Estimation of Donor T-Cell Content in Thymus Grafts

Deoxyguanosine was used in the culture medium to deplete donor thymocytes in the thymus allografts.^{37,38} To obtain an estimate of the number of donor thymocytes that remained in the thymus allografts, we cultured three thymuses with deoxyguanosine for two weeks. At the end of the culture period, the tissue was mechanically dissociated and viable thymocytes counted by flow cytometry. We found 0.5×10^6 to 1.0×10^6 thymocytes per gram of thymic tissue (weighed on the day of donation). Using the higher estimates and extrapolating to the size of the thymus grafts used in our patients, we concluded that each of our five patients received approximately 0.6×10^6 to 4.0×10^6 thymocytes per kilogram with their transplants. On the basis of this calculation, Patient 2, in whom predominantly donor T cells developed, received 8×10^5 thymocytes per kilogram.

DISCUSSION

The five patients we describe presented with profound cellular immunodeficiency and were classified as having the complete form of the DiGeorge syndrome.¹² Two patients (Patients 2 and 5) also had

features consistent with the CHARGE association, a rare combination that has been reported previously.^{8,12,33,36} All five patients had few or no detectable circulating T cells (indicated by the expression of CD3). PBMCs did not proliferate in response to mitogens. In Patients 3, 4, and 5, PBMCs stimulated with interleukin-2 produced vigorous proliferation, presumably due to the proliferation of natural killer cells. Only two of five patients were hemizygous for 22q11; that abnormality is not necessary for confirming the diagnosis of the DiGeorge syndrome.

After thymus transplantation, circulating T cells developed in increasing numbers in four patients (Patients 1, 2, 3, and 5). In Patients 2, 3, and 5, a substantial response to mitogens was observed on days 27, 28, and 30 after transplantation, respectively; it paralleled the increase in circulating T cells. This time course is similar to that in three reports of fetal thymus transplantation in which T-cell proliferative function increased two days,¹⁸ two weeks,¹⁶ and one month¹⁹ after transplantation. T-cell function in Patient 1 developed more slowly²³; normal responses to concanavalin A were detected only after eight months.

TRECs are the episomal circular DNA excision products of T-cell-receptor gene rearrangement. They are not replicated with cell division and are therefore diluted out during proliferation that is associated with antigenic stimulation. Thus, the presence of TRECs in peripheral T cells is thought to be a marker for cells that have recently emigrated from the thymus.³⁹ In Patient 5, we found a temporal correlation among T-cell proliferative function, lymphocyte phenotype, and level of TRECs. In contrast to our finding of an absence of proliferative function, an absence of TRECs, and an absence of CD45RA+CD62L+ T cells before thymus transplantation in Patient 5, six months after transplantation we detected increased proliferative responses to mitogens, increased percentages of CD45RA+CD62L+ T cells, and increased levels of TRECs in both CD4+ cells and CD8+ T cells (Fig. 1B). Remarkably, in regard to her normal T-cell function (Table 2), Patient 1 has continued to have CD45RA+CD62L+ T cells and TRECs, suggesting ongoing thymopoiesis (T-cell development in the thymus), more than five years after transplantation.

The T-cell function of many patients with partial DiGeorge syndrome can improve spontaneously,¹¹ but our data argue against spontaneous T-cell improvement in patients with complete DiGeorge syndrome. The TREC levels of Patients 1 and 5 were undetectable in the period immediately before and after transplantation. Patient 5, when tested for the presence of CD45RA+CD62L+ T cells before transplantation, had no detectable cells of this naive phenotype. Lastly, if the patients had had spontaneous improvement, the host T cells would most likely have rejected the allograft. Graft rejection was not

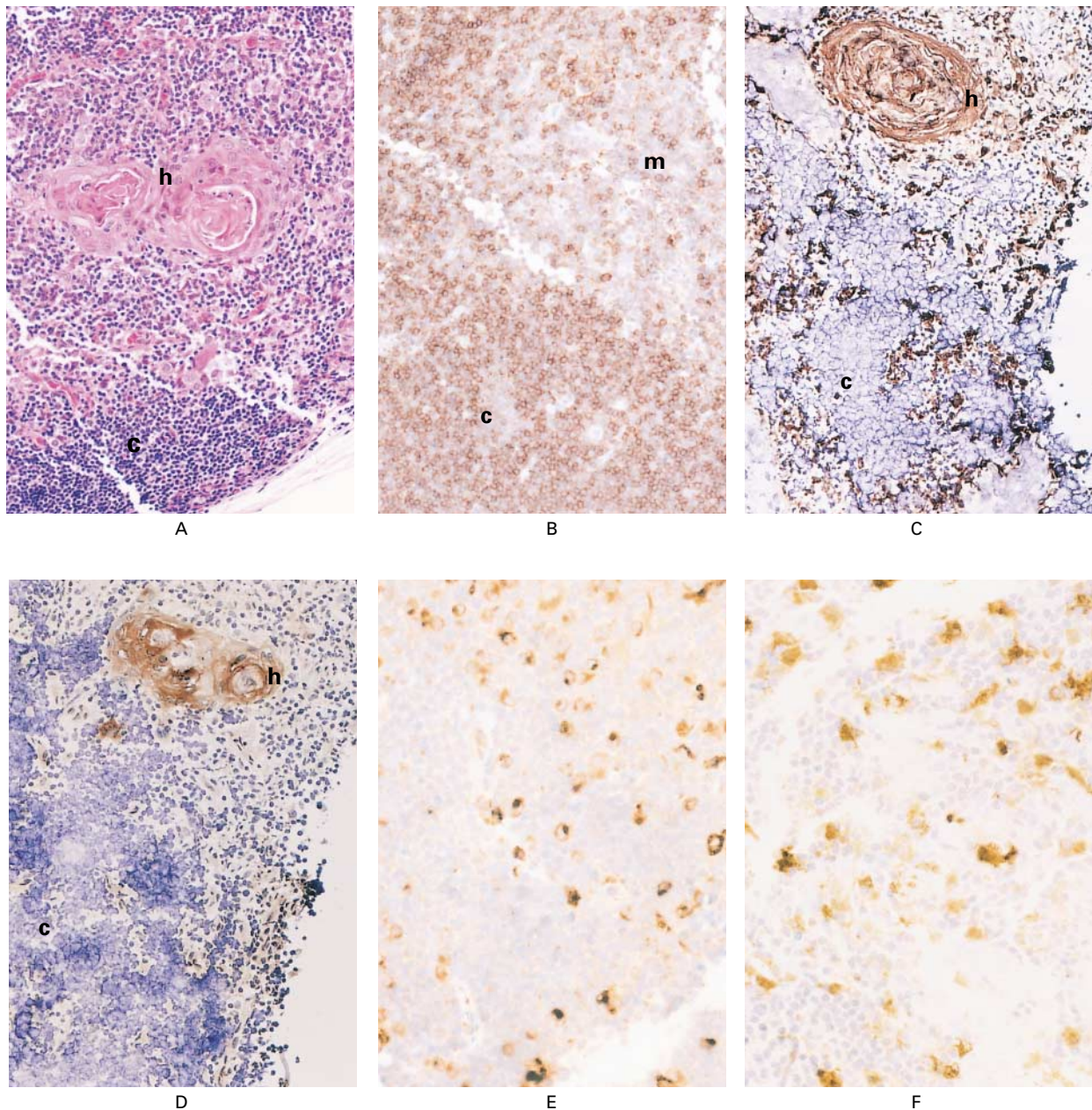


Figure 2. Biopsy Evaluation of the Thymus Graft in Patient 5.

In Panel A, hematoxylin and eosin staining shows normal cortical and medullary regions; the latter region contains a Hassall's corpuscle. Panels B through F show evaluations of immunohistochemical features. The brown color indicates antibody reactivity. In Panel B, CD1a reactivity is found on densely packed cortical thymocytes. In Panel C, cytokeratin reactivity is found in the Hassall's corpuscle and in epithelial cells (reticular pattern) in the cortex and medulla. In Panel D, the TE16 antibody reacts with the Hassall's corpuscle but not with other cells in the thymic epithelium. In Panel E, the KP-1 antibody reacts with cells that resemble macrophages. In Panel F, S-100 reactivity is consistent with the presence of dendritic cells. These antibody reactivity patterns are the same as those found in a normal thymus. Formalin-fixed tissue is shown in Panels A, B, E, and F, and frozen sections are displayed in Panels C and D. The abbreviation h denotes Hassall's corpuscle, c cortex, and m medulla. (Panels A through D, $\times 96$; Panels E and F, $\times 191$.)

found on biopsy of the allograft, however. Thus, we conclude that the reconstitution of T-cell function resulted from transplantation of the thymus tissue.

After transplantation, large numbers of circulating donor T cells were detected in Patient 2, but only small percentages of donor T cells were identified in Patients 3 and 5. No donor cells were detected by fluorescence in situ hybridization in Patient 1. Engraftment of donor T cells after thymus transplantation has not been reported previously in patients with the DiGeorge syndrome.

Although there was at least one full-haplotype mismatch between donor and host in all patients, no graft-versus-host disease or graft rejection was detected at the autopsy in Patients 2 and 3, in the biopsies of the thymic allografts in Patients 1, 3, and 5, or clinically in any of the patients. We estimated that the patients received up to 4×10^6 donor thymocytes per kilogram with the thymus transplant. In bone marrow transplantation, it is possible to see mild graft-versus-host disease at doses greater than 10^5 T cells per kilogram.⁴⁰ We speculate that the absence of graft-versus-host disease in our patients was related to the immaturity of the donor T cells in the thymus grafts. Alternatively, the culture period may have affected T-cell function or resulted in the loss of a subpopulation of cells.

Thymopoiesis is characterized by an ordered pattern of development of T-lineage cells in the thymus. When thymopoiesis is occurring, the evaluation of tissue sections with monoclonal antibodies shows cortical thymocytes coexpressing CD4, CD8, and CD1a and medullary thymocytes expressing either CD4 or CD8 but not CD1a. Immunohistologic evaluation of the thymic-allograft tissue did not reveal thymopoiesis in Patient 2 or Patient 3 but showed normal thymopoiesis in the thymus grafts in Patient 1²³ and Patient 5. Experiments in animals suggest that major-histocompatibility-complex matching is not necessary for immune reconstitution after transplantation of the thymus.⁴¹⁻⁴⁸ We continue to attempt partial matching for HLA-DR, because some matching might be helpful. It is not known whether the genetic basis of the thymic aplasia affects the development of thymopoiesis. Neither Patient 1 nor Patient 5 was hemizygous for 22q11. Another factor that may have affected thymopoiesis was the use of steroids. The use of steroids shortly before death in Patients 2 and 3 may have depleted the grafts of developing thymocytes. On the basis of our limited experience, we believe that risk factors for the failure of thymus transplantation include mechanical ventilation, cytomegalovirus infection, and steroid therapy.

Both Patient 1 and Patient 5 had populations of T cells present before transplantation that were oligoclonal on TCRBV analysis, that did not proliferate in response to mitogen stimulation (Tables 2 and 3 and Fig. 1A), and that were not associated with TRECs.

Thus, these early increases in oligoclonal T cells did not result from thymopoiesis, which would have produced detectable TRECs, but instead from extrathymic proliferation of preexisting extrathymically differentiated T cells.

Finally, our study shows success in using postnatal thymus tissue for transplantation. The abundance of postnatal thymus tissue as compared with the relative rarity of fetal thymus tissue makes this procedure readily available to children with complete DiGeorge syndrome. We recommend this procedure for patients with complete DiGeorge syndrome who have no T-cell proliferative responses to mitogens.

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