Thymus transplantation in complete DiGeorge syndrome: immunologic and safety evaluations in 12 patients

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Complete DiGeorge syndrome is a fatal condition in which infants have no detectable thymus function. The optimal treatment for the immune deficiency of complete DiGeorge syndrome has not been determined. Safety and efficacy of thymus transplantation were evaluated in 12 infants with complete DiGeorge syndrome who had less than 20-fold proliferative responses to phytohemagglutinin. All but one had fewer than 50 T cells/mm^3. Allogeneic postnatal cultured thymus tissue was transplanted. T-cell development was followed by flow cytometry, lymphocyte proliferation assays, and T-cell receptor Vβ (TCRBV) repertoire evaluation. Of the 12 patients, 7 are at home 15 months to 8.5 years after transplantation. All 7 survivors developed T-cell proliferative responses to mitogens of more than 100 000 counts per minute (cpm). By one year after transplantation, 6 of 7 patients developed antigen-specific proliferative responses. The TCRBV repertoire showed initial oligoclonality that progressed to polyclonality within a year. B-cell function developed in all 3 patients tested after 2 years. Deaths were associated with underlying congenital problems. Risk factors for death included tracheostomy, long-term mechanical ventilation, and cytomegalovirus infection. Adverse events in the first 3 months after transplantation included eosinophilia, rash, lymphade- nopathy, development of CD4⁻CD8⁻ peripheral T cells, elevated serum immunoglobulin E (IgE), and possible pulmonary inflammation. Adverse events related to the immune system occurring more than 3 months after transplantation included thrombocytopenia in one patient and hypothyroidism and alopecia in one other patient. Thymic transplantation is efficacious, well tolerated, and should be considered as treatment for infants with complete DiGeorge syndrome. (Blood. 2003;102:1121-1130)

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Introduction

DiGeorge syndrome is a congenital disorder caused by developmental defects in the third pharyngeal pouch and fourth pharyngeal arch.[1] As a result, defects are found in the thymus, heart, and parathyroid glands.[2,3] Approximately 90% of patients are hemizygous at chromosome 22q11.[4,5] Associated problems include gastroesophageal reflux, laryngomalacia, and speech delay.[3,6,7] Other terms used to characterize these patients include CATCH22 (cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, hypocalcemia, chromosome 22[8]) and 22q11.2 deletion syndrome.[9,10] DiGeorge syndrome can be found in conjunction with CHARGE association (coloboma, heart defect, agenesis choanae, retardation of growth or development, genital hypoplasia, and ear anomalies or deafness); these patients are rarely 22q11 hemizygous.[11-13]

Patients with DiGeorge syndrome are heterogeneous with respect to immune, cardiac, parathyroid, and other findings.[14,15] Most patients have some thymic function. They may be born with low T-cell numbers but have significant proliferative responses to mitogens and antigens[9] and normal antibody responses to immunization. T-cell numbers and function usually increase in these patients in the first years of life. It is rare for patients to have complete absence of the thymus.[16] These patients have "complete DiGeorge syndrome" and do not recover spontaneously.[15,16]

Complete absence of the thymus can only be inferred on the basis of laboratory data because imaging studies or physical examination of the mediastinum may not detect a small thymus located in an ectopic position. The laboratory findings that indicate thymic function include the coexpression of CD45RA and CD62L on peripheral blood T cells[17] and the presence of T-cell–receptor rearrangement excision circles (TRECs) in peripheral blood T cells.[18-20] TRECs are the episomes formed when T-cell–receptor variable (V), diversity (D), and joining (J) segments come together in the chromosome.[18-20] In this study, patients did not have evidence of thymic function. This was defined as having fewer than 50 naive T cells/mm^3 (normal > 1000/mm^3). If T cells were present in sufficient numbers to assay for TRECs, they had fewer than 100 TRECs/100 000 T cells (normal 10 000 TRECs/100 000 T cells).

It is possible for patients with complete DiGeorge syndrome to form T cells because there is no genetic defect preventing T-cell development. (This will be the subject of a future report.) These

T cells develop after a variable length of time; they have a limited T-cell receptor (TCR) repertoire; they do not coexpress CD45RA and CD62L; they do not have detectable TRECs; they may or may not respond to mitogens (M.L.M., unpublished data, November 1999-July 2002). The patients usually have a rash and lymphad-enopathy.[21] These clinical findings are likely related to lack of thymic education. The author refers to these patients as having "atypical" complete DiGeorge syndrome. All of the patients in this report had "typical" complete DiGeorge syndrome, with the possible exception of DIG001 who presented with rash 2 days prior to transplantation. The atypical form of complete DiGeorge syndrome is reviewed here to illustrate why the focus of the inclusion criteria is on naive T-cell numbers not total T-cell numbers.

This report summarizes a phase 1 trial conducted from 1993 to 2001 at one center that examined the safety of thymus transplanta-tion in 12 patients with complete DiGeorge syndrome. The excellent survival and immune outcome of this group suggest that thymic transplantation should be considered as treatment for the immune deficiency of patients with complete DiGeorge syndrome.

Patients, materials, and methods

Regulatory affairs

The protocol was reviewed by the Duke University Medical Center and University of North Carolina Institutional Review Boards and the Food and Drug Administration (FDA). Informed consent was obtained from the parents of the thymus donors and recipients.

Research subject inclusion and exclusion criteria

All research subjects had less than a 20-fold T-cell proliferative response to the mitogen phytohemagglutinin (PHA) and had fewer than 50 $CD3^+$ T cells/mm³ (cells $\times 10^{-6}$/L) coexpressing CD45RA and CD62L. An additional entrance criterion was the presence of cardiac defects, hypocalce-mia, and/or 22q11 hemizygosity. A total of 16 patients were screened for this study. Four subjects died prior to completion of screening or became too unstable for transplantation. Thus a total of 12 subjects with complete DiGeorge syndrome defined by the criteria above were treated with thymus transplantation under this protocol.

Thymus donor inclusion and exclusion criteria

Donors of thymic tissue were infants younger than 6 months of age who underwent corrective cardiac surgery for which thymus tissue was excised by the surgeon to expose the operative field. Consent for use of the discarded thymus in transplantation was obtained from the donor's parents. Donor testing for infectious agents followed FDA guidelines.[22] The donor's biologic mother was tested for HIV-1, HIV-2, human T-cell lymphotropic virus 1 (HTLV-1), HTLV-2, hepatitis B, hepatitis C, and syphilis. The donor was tested for cytomegalous virus (CMV), by urine culture, and Epstein Barr virus (EBV), by polymerase chain reaction (PCR) on the thymus tissue. Exclusion criteria included Down syndrome, autoimmune disease in the parents or siblings, or known 22q11 hemizygosity. Discarded blood left over in blood gas syringes was used for 22q11 testing, flow cytometry, and HLA class II typing.

Thymus tissue processing and grafting

Thymus processing was done as described[23] for the initial 5 patients. Processing has subsequently been conducted under current Good Tissue Practice.[24] In brief, thymus was brought to the laboratory in sterile fashion. It was sliced using a Stadie-Riggs hand microtome (Thomas Scientific, Swedesboro, NJ)[25] into slices 0.5 mm thick. The slices were placed on Millipore filters on surgical sponges in Petri dishes in thymus organ medium. This medium consists of F12 nutrient mixture (Ham; Gibco, Grand Island, NY), with 1.36 mM 2-deoxyguanosine (Sigma, St Louis,

MO), 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Gibco), 2 mM L-glutamine (Gibco), 10% fetal bovine serum (Gibco), 100 μg/mL streptomycin sulfate (Gibco), 1 μg/mL gentamicin (Gibco), and 100 μg/dL Amphotericin B (GensiaSicor, Irvine, CA). Thymocytes that fell into the medium during slicing were used for HLA typing. The slices were maintained in a 5% CO_2 incubator at 37°C. The medium was changed daily. Cultures for bacteria, fungus, and mycoplasma, and gram stains were performed on days $-7, -3$ and on the day of transplantation. A limulus amebocyte lysate (LAL) assay was performed on supernatant the day prior to transplantation to detect excessive lipopolysaccharide (LPS). The dose of LPS per kg of recipient body weight had to be less than 5 endotoxin units/kg. (The amount of endotoxin delivered was assumed to be the amount in the supernatant.) All slices were measured the day prior to transplantation to obtain an estimated dose. For transplantation, the thymus slices were placed bilaterally into both quadriceps muscles. After DIG006 underwent allograft biopsy at 2.1 months, it was decided to make the biopsy time uniform at approximately 3 months. The only major deviation from this plan was the biopsy in DIG012 that was delayed until 7.2 months for medical reasons. Thymus transplantation and allograft biopsies were done as open procedures in the operating room under general anesthesia.

Immune testing, TRECs, and graft biopsies

Standard assays were performed as described.[23] The presence of recent thymic emigrants was assessed by flow cytometry and the signal joint TREC assay.[26] Naive cells were defined as those expressing CD3 plus CD45RA and CD62L.[17] The TREC assay[23,26,27] and the immunohistochemi-cal evaluation of allograft biopsies[23,28,29] were done as described. Control data for TREC levels in children have been reported.[30]

Evaluation of T-cell repertoire diversity

The immunoscope technique was used to evaluate TCR repertoire diver-sity[31-33] by amplification of rearranged TCR Vβ (BV) chain cDNAs. Each BV family was assessed individually using forward BV-specific primers and reverse constant region (BC)–specific primers. The products were visualized by an elongation reaction using a nested fluorescent BC primer. The elongation products containing the complementarity determining region 3 (CDR3) were separated on a sequencing gel. The results were analyzed using the Gene Scan software (Applied Biosystems, Foster City, CA) and are shown as graphic distribution of size peaks centered on a peak corresponding to a CDR3 of 8 to 10 amino acids. Normal profiles have a Gaussian-like distribution of an average of 6 to 8 peaks per BV family (polyclonal Gaussian). The peaks are spaced by 3 nucleotides. Comparisons with size standards shows that they correspond to in-frame transcripts. Alterations of the normal profile, resulting in a shift of the center of CDR3 distributions or in a disruption of the normal peak distribution due to clonal T-cell expansions, results in a polyclonal skewed profile. Finally, oligo-clonal T-cell expansion results in profiles of no more than 4 peaks per BV family. Data are expressed as percent of oligoclonal, polyclonal skewed, or polyclonal Gaussian BV families per total number BV families amplified from a sample.

TRECs

The evaluation of TRECs was done per Douek and colleagues.[23,26] In brief, quantification of TRECs in peripheral blood mononuclear cells (PBMCs), total $CD3^+$ cells, or sorted $CD4^+$ and $CD8^+$ T cells was performed by real-time quantitative PCR using the 5'-nuclease (TaqMan) assay with an ABI7700 system (Perkin-Elmer, Norwalk, CT). A standard curve was plotted and TREC values for samples were calculated by the ABI7700 software. Samples were analyzed in duplicate.

Results

The patient characteristics at presentation are shown in Table 1. Eleven of the 12 patients had fewer than 50 total T cells/mm³. All had fewer than 50 T cells of naive phenotype/mm³. T-cell function

was extremely low; all patients had less than a 20-fold response to PHA. Of note, 6 patients were normal for chromosome 22q11. Five patients had CHARGE association. Table 2 provides the HLA typing of the thymus donors and recipients. Matching for HLA was not a criterion for transplantation.

The Kaplan-Meier survival curve is depicted in Figure 1. The survival rate is 58% with 15 months to 8.5 years of follow-up. The development of immune function was followed closely. Figure 2 shows the increases in CD3 numbers and T-cell subsets with time. Several patients had early (0-4 months) oligoclonal proliferation of T cells. The final T-cell counts of individual patients after one year ranged from 479/mm^3 (the mean of values for DIG001) to 1580/mm^3 (the mean for DIG005). All patients have more CD4 than CD8 cells.

All surviving patients developed T-cell proliferative responses to mitogens (Figure 3A) and anti-CD3 (not shown). All patients developed a proliferative response to tetanus toxoid (Figure 3B). The tetanus response of DIG001 at age 7 may be low because she had not been immunized for 3 years.

The 3 oldest patients who have been taken off intravenous immunoglobulin (IVIG) make specific antibody responses to tetanus toxoid. DIG001, DIG005, and DIG006 have been immunized to pneumovax and all respond appropriately for their age (data not shown).

The appearance of T cells in the periphery and the development of T cells was correlated with the appearance of markers for naive T cells. Cryopreserved samples were used for the early TREC values for DIG001. Figure 4 shows the increase in TRECs after thymus transplantation.

Table 1. Clinical and immunologic findings prior to transplantation and procedures required for clinical care

ID no., age at tx*	22q11†, CHARGE status	Clinical findings at presentation	Initial T-cell count‡, age	PHA response‡, (bkg), age
DIG001§, day 90	22q11 normal	PDA, hypocalcemia (with seizure), GER, single left kidney	1/mm^3‖, day 36	1 457 cpm (1 045 cpm) day 71
DIG002, day 96	22q11 normal CHARGE	PDA, tracheomalacia, GER, ear anomaly, hypocalcemia, coloboma, microphallus, cleft lip and palate	10/mm^3, day 23	568 cpm (654 cpm) day 23
DIG003, day 51	22q11 hemi	Aortic narrowing, abnormal vessels, dilated coronary sinus, omphalocele, malrotation, malformed ear	0/mm^3, day 33	8 674 cpm (9 965 cpm) day 33
DIG004, day 127	22q11 hemi	ASD, PDA, right aortic arch, cleft soft palate, abnormalities of digits, hypocalcemia, severe GER, tracheomalacia, CMV infection, chronic ventilation, use of oscillator	0/mm^3, day 126	1 093 cpm (1 214 cpm) day 62
DIG005, day 63	22q11 normal CHARGE	Microphallus with undescended testes, laryngomalacia, ovoid left kidney, transient hypocalcemia, right vocal cord adduction paresis after surgery, PDA, left SVC, dilated coronary sinus, bilateral coloboma, hearing deficit, nasopharyngeal reflux with aspiration, development delay	337 CD4 T cells/mm^3; and 0 CD8 cells (3 naive CD4 T cells/mm^3), day 59¶	15 079 cpm (964 cpm) day 31; and 1864 cpm (573 cpm) day 44#
DIG006, day 33	22q11 hemi	TOF, right aortic arch, hypocalcemia, malrotation	16/mm^3, day 27 (note: subsequent 10 tests had 0/mm^3 T cells)	360 cpm (362 cpm) day 5
DIG007, day 133	22q11 hemi	IAA type B, PDA, VSD, hypocalcemia, laryngomalacia, GER, episodes of sepsis, right renal agenesis, grade I hydronephrosis on left, hernias, poor swallow	0/mm^3, day 115	305 cpm (128 cpm) day 119
DIG009, day 75	22q11 hemi	Hypocalcemia, aberrant right subclavian artery	0/mm^3, days 17 and 42	Not done
DIG010, day 53	22q11 normal CHARGE	GER, PDA, choanal atresia, hearing loss, bilat colobomas, hydoureteral reflux grade 4, hydronephrosis, microphallus, undescended testes	22/mm^3, day 43	426 cpm (219 cpm) day 43
DIG011, day 67	22q11 normal CHARGE	Bilat coloboma, AV canal, complete right sided cleft lip and palate, developmental delay, decreased hearing, hypocalcemia, inguinal hernias, GER, partial agenesis of corpus callosum, single left kidney, grade I hydronephrosis	0/mm^3, day 41	204 cpm (340 cpm) day 41
DIG012, day 82	22q11 normal	Truncus, hypocalcemia, hemivertebra T10-11 on left, GER	0/mm^3, day 27	1 701 cpm (476 cpm) day 32
DIG016, day 107	22q11 hemi CHARGE	PDA, TOF, hypoplastic right and left PA, VSD, hypocalcemia, single right kidney, hydroureteronephrosis, decreased hearing, GER	0/mm^3, days 41 and 86	1 318 cpm (677 cpm) day 41

tx indicates transplantation; PHA, phytohemagglutinin; bkg, background; PDA, patent ductus arteriosis; GER, gastroesophageal reflux; cpm, counts per minute; hemi, hemizygous; ASD, atrial septal defect; CMV, cytomegalovirus; SVC, superior vena cava; TOF, tetralogy of Fallot; IAA, interrupted aortic arch; VSD, ventricular septal defect; AV, atrial ventricular; and PA, pulmonary atresia.

*The ages in this table are days of life.

†Occasional DiGeorge syndrome patients have been identified who are hemizygous for 10p13.[34] These patients can always be detected by high-resolution chromosome banding (P. Scambler, personal e-mail communication, April 2002), which was done on each patient who was not 22q11 hemizygous. No patient was hemizygous for 10p13.

‡Normal values are as follows: normal T-cell count for age 2 days to 11 months is 1700 to 3600/mm^3 for the 25th to 75th percentile[35]; the mean control PHA response for these assays was 223 701 cpm (± 1 SD was 144 954 cpm to 345 226 cpm). The background represents the cpm of cells incubated with medium only.

§Patients DIG001–DIG005 were reported previously.[23,28,36]

‖This data point was obtained from the referring hospital.

¶The T-cell count is given from day 59 of life since this is the first day that naive cells were examined. Earlier total T-cell counts were similar. No genetic testing was done on these T cells prior to transplantation. On day 63 after transplantation 16% of the circulating T cells were female (donor). By 2 years, all sorted T cells were male. An additional less sensitive test (sex chromosome analysis of PBMCs) showed only male cells on day 79 after transplantation.

#The PHA response reported is the first one obtained. Although it looks higher than that of the other patients, on that same day the concanavalin A (ConA) response was 749 cpm with a background of 964 cpm. Two weeks later, the PHA response was only 1269 cpm with a background of 573 cpm. The ConA response on this date was 1864 cpm with a background of 573. Thus, this patient is similar to the others in lack of T-cell function.

Table 2. HLA typing of donor and recipients

Recipient	HLA-A	HLA-B	HLA-DR	Donor	HLA-A	HLA-B	HLA-DR
DIG001	2*	7	*1302	MLM5	2	40	*1501
		62	*0401		24	44	*1301
DIG002†	29	44	*0701	MLM18	29	44	*0701
	11	55	*1401		31	47	*0405
DIG003	1	35	10	MLM93	2	7	7
	1	44	15		3	7	15
DIG004	2	7	8	MLM96	1	8	*0704
		28	13		2	18	
DIG005†	*02xx	*0702-08	*1201	MLM107	*02xx	*0702-08	*1201
	*0301-03	*1301-03	*0301		*02xx	*4402-05	*0101
DIG006	2	45	*1503	MLM120	23	*4501	*1101
	34	35	*0302		23	*5301-02	*1302/34
DIG007	2	44	7	MLM129	23	*1503	*0701
	33	51	14		30		*1302
DIG009	*3201	*3801	4	MLM136	*2301-05	*35xx	*0301
	24	*4002	14		*3001-07	*53xx	*1302
DIG010	3	51	1	MLM137	*0104-06	*07xx	*0101
		35	4		*24xx	*55xx	*1501
DIG011	2	7	15	MLM138	*02xx	51	*0701
	33	51	13		*3001-07	*1301-03	*1301/35
DIG012	*2301-06	*35xx	*0405	MLM141	*02xx	*44xx	7
		*4501-03	*0804		*0301-06	*5001-04	9
DIG016	*03	*51	*04	MLM146	*0101-07	*07	*0301
	*31	*4001/07/22N/25(60)	*13		*0301-06	*08	*1101
				MLM147‡	Not done	Not done	*0301/16
							*1501

*Numbers without asterisks refer to serologic typing. Numbers preceded by asterisks indicate PCR based typing. Blanks indicate a null allele or homozygosity for an allele.
†The haplotype matching was by chance. The donor and recipient were not related. xx indicates that the specific allele was not typed.
‡DIG016 was given 2 thymuses. The HLA typing on MLM147 was not completed.

Figure 5 shows the increases in T cells coexpressing the markers of recent thymic emigrants, namely CD45RA and CD62L. This test was not available when DIG001 was given a transplant. Flow cytometry for CD45RA and CD62L has only been done on this patient for the past 3 years. Normal values have been reported for the absolute number of cells expressing CD3, CD4, and CD45RA.[37] CD3 cells coexpressing CD45RA and CD62L are always fewer in number than the those bearing CD45RA with or without CD62L. Thus, the number of naive T cells in these patients after transplantation is lower than the number seen after birth. It is similar to the number seen in healthy adults.

The graft biopsies revealed viable thymic epithelium in DIG001, DIG005, DIG006, DIG009, DIG011, and DIG012. Thymus allograft was not found on biopsy in DIG007 and DIG010. The small biopsy obtained in each of these latter 2 cases contained fat not thymus. It is thought that if additional pieces of tissue had been obtained, the allograft would have been found. To enhance correct identification of allograft, the subsequent biopsies were immediately placed in formalin. Floating pieces contain fat and are rejected.

The determination that thymopoiesis was present required 2 findings: (1) cortical thymocytes with phenotype of CD3^{+}, CD1a^{+}, Ki-67^{+} (Key et al[38]); and (2) reticular keratin-positive epithelium.

Figure 1. Kaplan Meier curve of 12 patients that received transplants. Patient survival is plotted.

Patients DIG001, DIG005, DIG006, DIG009, DIG011, and DIG012 had these findings. The micrographs for DIG009 and DIG012 resembled the illustrations previously published for DIG001 and DIG005.[23] The biopsies of DIG006 and DIG011 did not contain medullary areas or Hassall bodies. The biopsy in DIG006 was performed earlier than all the others (at 2.1 months), which may explain this difference. Patient DIG011 had the graft placed while in congestive heart failure. The week after thymus transplantation, the patient had corrective heart surgery with circulatory arrest. This stress on the thymus graft may have been detrimental to engraftment.

The immunoscope technique was used to evaluate TCRBV repertoire (Figure 6A-C). Of note is the normalization of immunoscope profiles by 8 to 12 months after transplantation in 6 of 7 surviving patients. Data from both an early and late time point are shown for DIG012 in Figure 6A-B to illustrate the early oligoclonality and later polyclonality. Summary data from all patients are shown in Figure 6C. Patients DIG001, DIG005, DIG006, and DIG007 were not studied at an early time point because the technique was not available at that time. Patients DIG009, DIG010, and DIG012 had markedly oligoclonal TCRBV profiles when tested at less than 120 days after transplantation (Figure 6C). All 3 improved at the subsequent time point having profiles similar to patients DIG001, DIG005, DIG006, and DIG007. Although patient DIG010 had an abnormal immunoscope profile at 12 months, his repertoire has improved markedly from that of 91 days, increasing from 0% to 33% polyclonal Gaussian TCRBV families and decreasing from 67% to 5% oligoclonal families.

Patients experienced many adverse events before and after transplantation (Table 3). Infections were frequent (Table 4) as is expected in this immunodeficient population. The patients had many infections in the first 100 days after transplantation (prior to development of T-cell function) and fewer after that time. The
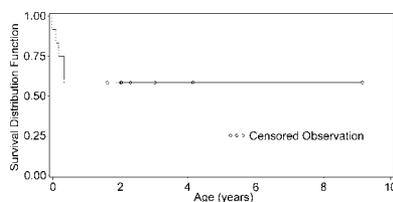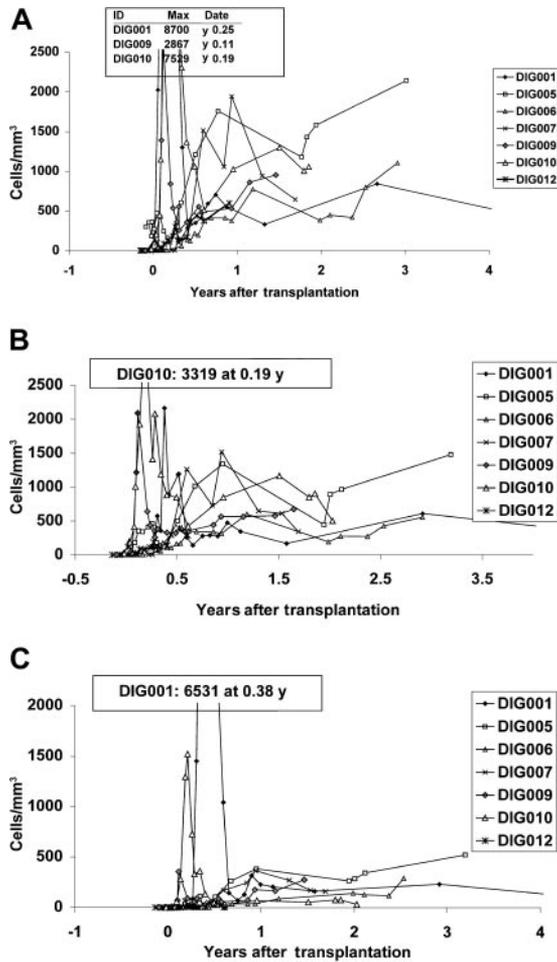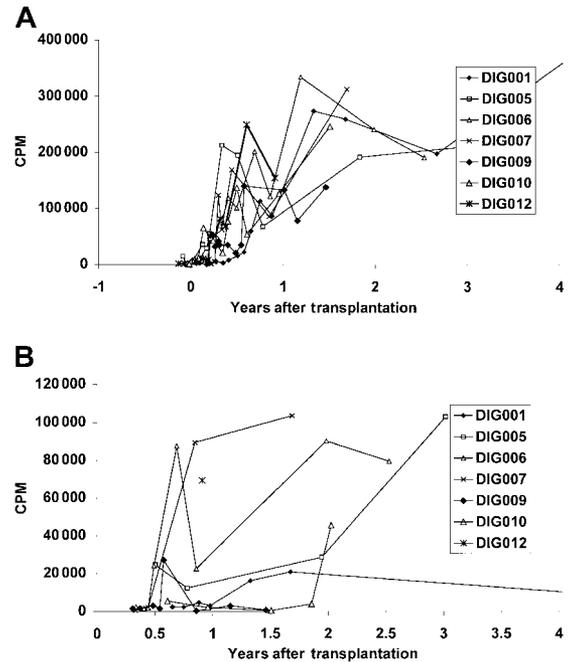
Figure 2. T-cell counts in surviving patients. (A) CD3 counts, (B) CD4 counts, and (C) CD8 counts. The Y-axis maximum in each graft does not show the peak from early oligoclonal proliferations. The early oligoclonal peaks for patients DIG001, DIG009, and DIG010 are indicated on the grafts. Only 4 years of data are depicted because only one patient, DIG001, is more than 4 years from transplantation. The most recent CD3, CD4, and CD8 counts for patient DIG001 are 337/mm³, 178/mm³, and 112/mm³ from 8 years after transplantation, respectively. The normal CD3 T-cell count for age 2 days to 11 months is 1700 to 3600/mm³, for age 1 to 6 years is 1800 to 3000/mm³, for age 7 to 17 years is 1400 to 2000/mm³.[35] The normal CD4 T-cell count for age 2 days to 11 months is 1700 to 2800/mm³, for age 1 to 6 years is 1000 to 1800/mm³, for age 7 to 17 years is 700 to 1100/mm³.[35] The normal CD8 T-cell count for age 2 days to 11 months is 800 to 1200/mm³, for age 1 to 6 years is 800 to 1500/mm³, for age 7 to 17 years is 600 to 900/mm³.[35] These ranges all reflect the 25th to 75th percentiles.[35]

adverse events after 100 days were often not primarily infectious complications secondary to poor immune function, but were related to other congenital anomalies. For instance, DIG001 had pyelonephritis requiring hospitalization; however, she is susceptible to renal problems because of her single kidney. DIG012 was hospitalized for diarrhea and low calcium; however, it was the low calcium that made the admission necessary. Other patients had infections of central lines (DIG010) or gastric tube sites (DIG006), which often occur in immune-competent children. The infection that seemed to be the most difficult for the posttransplantation DiGeorge syndrome infants was respiratory syncytial virus (RSV). Hospitalizations were needed to treat RSV in both DIG006 and DIG007. Taking all of the adverse events into account, however, these patients have done remarkably well after the development of T-cell function.

There were a number of other adverse events. Calcium problems were notable resulting in several seizures and one death. Autoimmune disease was seen in 2 patients. DIG006 had 2


Figure 3. Proliferative T-cell responses in surviving patients. Proliferative responses to (A) PHA and (B) tetanus toxoid. As in Figure 2, only 4 years of follow-up are shown. The most recent PHA response for patient DIG001 is 223 303 cpm at 8 years after transplantation. The most recent tetanus toxoid response for patient DIG001, at 7 years, had fallen to background levels. The mean PHA response of the normal control assay done in parallel with these assays (n = 77) was 162 249 cpm (the range including 1 SD was 67 507 cpm to 389 958 cpm). The mean tetanus toxoid response of the healthyl control individual run in parallel with these assays (n = 27) was 26 161 cpm (the range including 1 SD was 6597 cpm to 103 750 cpm). The mean medium background for the patients' PHA assays (n = 87) was 361 cpm (the range including 1 SD was 153 cpm to 853 cpm). The mean patient background for the tetanus toxoid assays (n = 36) was 1221 cpm (the range including 1 SD was 402 cpm to 3717 cpm).

episodes of thrombocytopenia at days 313 and 466, and DIG007 developed hypothyroidism on day 427 and alopecia on day 838 after transplantation. DIG010 developed large numbers (3046/mm³) of double-negative T cells on day 77 after transplantation; this resolved without any therapy. Half of these T cells expressed TCRαβ and half expressed TCRγδ. They were oligoclonal as assessed by immunoscope (data not shown). The most important risk factors for death were prolonged intubation, tracheostomy, and CMV infection.
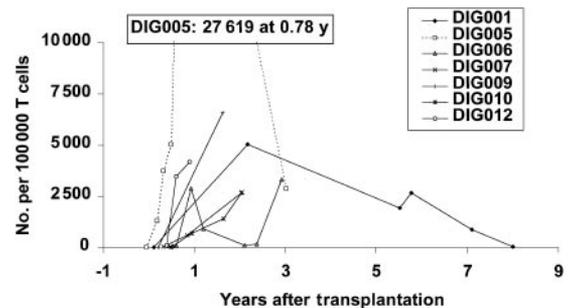
Figure 4. TREC values after transplantation. All data points in surviving patients are shown. TRECs are expressed in number per 100 000 cells. Normal values in infants are approximately 10 000/100 000 cells.[30] CD3 TRECs are reported except as stated here to indicate CD4 and CD8 TRECS were assayed separately.[3] The early time points for DIG001 and DIG005 are CD4 TRECs except the last data point for each. The CD4 TREC data for patients DIG001 and DIG005 have previously been reported.[23] For DIG006 and DIG007, all are, except the last 3 and 2 data points, respectively, CD4 TRECs.
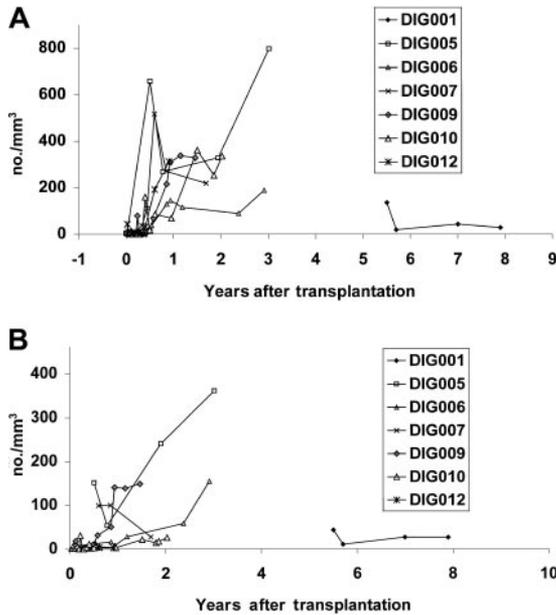
Figure 5. Naive T-cell numbers after transplantation. The numbers of (A) CD4^{+} T cells and (B) CD8^{+} T cells expressing CD45RA and CD62L are reported. For DIG009, the expression of CD45RA only is used (this patient's follow-up is at another center that does not use CD62L). The CD45RA and CD62L antibodies were not used in DIG001 at early time points. The normal expression of CD45RA on CD4 and CD8 T cells has been reported.[37] For age 6 months the number ranged (based on 11 infants) from 1900 to 4900/mm³; for age 12 months, the range was 1100 to 4300/mm³; for adults (based on 9 individuals), the range was 100 to 800/mm³. For cells expressing CD3, CD8, and CD45RA, the range at 6 months was 500 to 1900/mm³; the range at 12 months was 600 to 1300/mm³; the range for adults was 100 to 600/mm³.

Multiple patients developed either a rash resembling eczema (DIG001, DIG006, DIG009, DIG010, DIG011, DIG012), eosinophilia (DIG009), high immunoglobulin E (IgE; DIG006, DIG011), and/or wheezing or respiratory symptoms (DIG003, DIG011) when T cells first developed. The rash was often associated with T cells in the skin and with circulating oligoclonal T cells in all cases. See Figure 7 for a skin biopsy from patient DIG009 demonstrating T-cell infiltration of the dermis. This biopsy is similar to those from the other patients. All rashes were biopsied except for that in DIG010 and a minor rash in DIG011. None were consistent with graft-versus-host disease. The diagnosis in all patients was spongiotic dermatitis. The eosinophilia in DIG009 and high IgE and respiratory symptoms in DIG011 were treated with parenteral steroids (1-2 mg methylprednisolone/kg/d for 1-2 weeks followed by a taper).

Discussion

The projected survival for patients with complete DiGeorge syndrome without treatment is approximately 27% at 1 year and 0% by 2 to 3 years based on historic controls (M.L.M., unpublished).[16] The survival of 58% of patients (Figure 2) is remarkable for this group of DiGeorge syndrome patients. We compared the survival rate with that of infants with severe combined immunodeficiency (SCID) treated with bone marrow transplantation. One would expect that it would not be possible for DiGeorge syndrome patients to be able to achieve the same survival rate as that for SCID since DiGeorge syndrome patients have many more congenital problems. In one of the largest published series of SCID patients, survival after bone marrow transplantation was 75% to

80%.[39] None of the SCID infants had major heart defects, required tracheostomies, or had severe hypocalcemia problems, which were the etiologies for the deaths reported in this series. Thus, the 58% survival reported here is likely close to the highest survival rate possible for this group of patients.

Thymus transplantation compares favorably with other forms of treatment for the immune deficiency associated with complete DiGeorge syndrome. For bone marrow transplantation and peripheral blood transfer from HLA-identical siblings and for unrelated cord blood transplantation, the mechanism of immune reconstitution is adoptive transfer of mature T cells. Bone marrow transplantation using HLA-identical sibling donors has been reported in 2 patients.[40,41] One has been lost to follow-up. The other is living at home without infections. Transfer of peripheral blood mononuclear
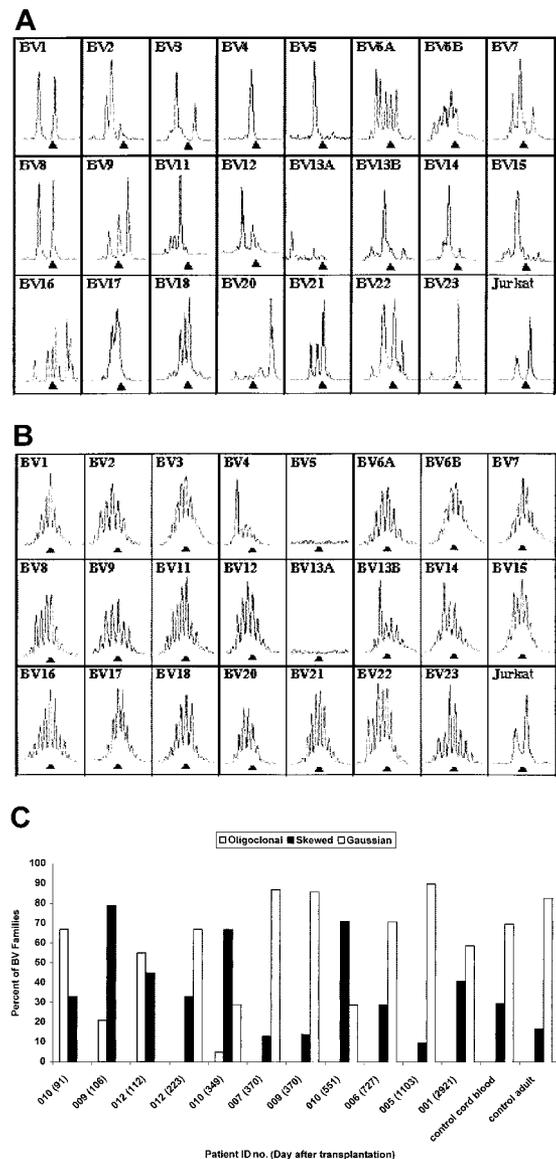
Figure 6. Immunoscope profiles of TCRBV repertoire. (A) Immunoscope profile for DIG012 on day 112 after transplantation. (B) Immunoscope profile for DIG012 at day 223 after transplantation. (C) Bar graph showing immunoscope TCRBV data for all surviving patients. The patient identification number and day after transplantation are given for each sample. Profiles from a control cord blood sample and a control adult sample are included. Each TCRBV family is graded as oligoclonal (eg, panel A, BV4), polyclonal skewed (eg, panel A, BV16), or polyclonal Gaussian (eg, panel B, BV6A).

Table 3. Adverse events and outcome

ID no.	Immune adverse event	Other adverse event	Outcome
DIG001	Rash, lymphadenopathy for 3 mo after transplantation, treated with topical steroids and a brief unsuccessful trial of interferon-gamma	Mild asthma (medications available as needed, oral steroids once a year), continues to require calcitriol	Age 9 years, in fourth grade, doing well. On calcitriol, free of infection.
DIG002	Not applicable	Intubation for stridor, respiratory collapse, tracheostomy for laryngomalacia	Death from intracerebral hemorrhage on d 66 after transplantation
DIG003	Possible respiratory inflammation when T cell developed, treated d 35 after transplantation with 30 mg/kg/d methylprednisolone $\times$ 3 d	Not applicable	Death from sepsis and respiratory failure on d 130 after transplantation
DIG004	Not applicable	Not applicable	Death from sepsis and respiratory failure 45 d after transplantation
DIG005	Not applicable	Not applicable	Age 3.5 years, living at home with severe developmental delay, walking, no speech, hearing aids, glasses, sign language, tube feeding. Free of infection.
DIG006	Mild skin rash 4 mo after transplantation (treated with topical steroids), thrombocytopenia $\times$ 2 (d 313 and 466) treated with Rh$_O$(D) immunoglobulin containing IgG anti-D (anti-Rh) and IVIG; IgE 519 and 568 IU/mL at the 2 times of thrombocytopenia	Mild nephrocalcinosis d 36, seizure from hypocalcemia (d 319), admission for FTT d 432, RSV admission d 516, mild hearing loss	Age 27 months, doing well, speech delay, eating by mouth. Free of infection, on calcium and calcitriol.
DIG007	Hypothyroidism d 427 treated with thyroxine, alopecia d 838 treated with betamethasone foam	Episodes of sepsis (d -74, $+72$, $+116$), continuing aspiration on swallow studies, d 334; mild bilateral hearing loss secondary to fluid documented d 420; hospitalized d 795 for 5 d for RSV, parainfluenza virus and rotavirus	Age 22 months, doing well, slight developmental delay, speech delay, mainly tube feeding. On trimethoprim/sulfamethoxazole, IVIG, calcium, calcitriol, free of infection.
DIG009	Rash on d 22, eosinophilia requiring steroids at d 44 (2 mg/kg/d solumedrol $\times$ 2 wk then taper)	Fever d 40, febrile seizure at d 359 after thymus transplantation, treated as outpatient with oral antibiotics	Age 17 months, doing well, slight developmental delay, speech delay, on IVIG, calcium, calcitriol, free of infection
DIG010	Rash starting 1 mo after transplantation, double-negative T cells to 3000/mm^3 2 months after transplantation with adenopathy, eosinophilia to 3456/mm^3, topical steroids used; all resolved	Unilateral vocal cord paralysis after cardiac surgery, rotavirus d 118, occasional episodes of otitis, hearing deficit d 202	Age 18 months, doing well, moderate developmental delay, speech delay, tube feedings, hearing aids, on trimethoprim/sulfamethoxazole, IVIG, free of infection
DIG011	Event d 77 after transplantation with increased ICP, high IgE to 14 400 IU/mL, poor neurologic status, worsening respiratory status, resolved with low-dose steroids (1 mg/kg/d methylprednisolone)	Subglottic stenosis, long ventilation needed secondary to CHF, tracheostomy because of long-term ventilation	Sepsis leading to death d 132 after transplantation
DIG012	Rash at d 84 (resolved with topical steroids)	Heart failure on d 9 of life with pH of 6.6; nephrocalcinosis, multiple episodes of hypocalcemia	Age 12 months, doing well, on trimethoprim/sulfamethoxazole, IVIG, calcium and calcitriol, free of infection, speech delay, tube and oral feeding
DIG016	Not applicable	Seizures from hypocalcemia prior to admission	Death from hemorrhage due to calcium accretion in IVC that ruptured during abdominal surgery on day of transplantation

IVIG indicates intravenous immunoglobulin; FTT, failure to thrive; ICP, intracranial pressure; CHF, congestive heart failure; and IVC, inferior vena cava.

cells has been reported for 2 patients (although T-cell numbers are < 500/mm^3).[42,43] However, 2 other unpublished cases did poorly with early pulmonary inflammation leading to the death of one patient (continued personal communication, Dr Prescott Atkinson, University of Alabama at Birmingham, January 2000-January 2002) and the requirement of high doses of steroids and cyclosporin in the other.

The latter patient developed unrelenting life-threatening autoimmune disease and died of sepsis 1.5 years after the T-cell treatment. Unrelated cord blood transplantation has been performed in one patient (continued personal communication, Dr Richard I. Schiff, Miami Children's Hospital, FL, January 2002). Although initially chimerism was achieved, the patient has fewer than 100 T cells/mm^3 after 2 years. Thus, the experience, safety profile, and immune outcomes of thymus transplantation compare favorably with those associated with other treatments for the immune deficiency of complete DiGeorge syndrome.

The immune outcomes after thymic transplantation are excellent. Patients have T-cell counts from 337 to 2140/mm^3 (Figure 2)

with excellent proliferative T-cell function (Figure 3). All have antigen responsiveness as well. Three of 3 transplant recipients surviving longer than 2 years also make specific antibodies in response to immunization. It is important to note, however, that most patients, although having normal proliferative function, do not have normal T-cell numbers at this time. Only by following the patients through time will the clinical significance of the low T-cell numbers be determined.

Markers of recent thymic emigrants were evaluated. The TREC numbers increased after thymus transplantation, usually to levels less than those seen in healthy newborns (which would be 10 000 TRECs per 100 000 CD3$^+$ T cells).[30] The oldest patient has very low TRECs (20/100 000 CD3 cells) at 8 years of age. This may reflect a diminution of function of the transplanted graft. This resembles the situation in patients with SCID after bone marrow transplantation.[30] These SCID patients have very small thymuses. They apparently function in the development of T cells, but the TREC values decrease with time, possibly reflecting early

Table 4. Infections after transplantation

| ID no. | Date of transplantation | Months | | | | Years |
		1-3	4-6	7-12	13-24	2-10
DIG001	1993	Blood: *Staphylococcus epidermidis, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium* CVL site: *Pseudomans aeruginosa, Staphylococcus epidermidis*; Urine: *Klebsiella oxytoca*	Blood: *Staphylococcus epidermidis, Staphylococcus aureus* CVL site: *Pseudomans aeruginosa* Stool: rotavirus	None	None	Pyelonephritis: in hospital 5 d
DIG002	1994	Blood: *Staphylococcus epidermidi* Urine: *Candida parapsilosis* ETS: Klebsiella Death from intracranial hemorrhage	Not applicable	Not applicable	Not applicable	Not applicable
DIG003	1997	Blood: *Enterococcus faecalis, Candida parapsilosis, Staphylococcus epidermidis* Eye: *Pseudomonas aeruginosa, Staphylococcus epidermidis* ETS: *Pseudomonas aeruginosa* Urine: *Candida parapsilosis*	Blood: enterococcus; ETS: methicillin-resistant staphylococcus Death from infection	Not applicable	Not applicable	Not applicable
DIG004	1998	Blood: *Enterococcus faecium, Staphylococcus epidermidis, Candida albicans, Candida glabrata,* Vancomycin-resistant enterococcus (VRE), alpha hemolytic streptococcus NP: CMV Urine: enterococcus (VRE) Death from infection	Not applicable	Not applicable	Not applicable	Not applicable
DIG005	1998	None	None	None	None	None
DIG006	1999	None	None	None	Hospitalization for RSV	Minor infection of G-tube site treated with oral antibiotics
DIG007	2000	Fever × 1 requiring IV antibiotics (culture negative)	Fever × 1 requiring IV antibiotics (culture negative)	None	ER: fever and diarrhea (culture negative) URI × 1 Hospitalized: RSV plus parainfluenza plus rotavirus 5 days	Hospitalization a few days with diarrhea
DIG009	2000	Fever (culture negative)	None	Fever to 38°C and seizure treated with augmentin	None	None
DIG010	2000	Thrush, eye discharge, fever to 39°C	Hospitalization for rotavirus, draining ear	Otitis	Otitis, portacath infection: enterobacter and enterococcus, persistent cough (secondary to aspiration)	None
DIG011	2000	NP: RSV Blood: *Staphylococcus epidermidis* BAL: enterococcus species Urine: enterococcus	Fevers, persistent (no positive cultures) Death from sepsis	Not applicable	Not applicable	Not applicable
DIG012	2001	Blood: *Stenotrophomonas maltophilia*	None	Hospitalization: bronchitis	Hospitalization: diarrhea and low calcium	None
DIG016	2001	Death on day of transplantation (from hemorrhage)	Not applicable	Not applicable	Not applicable	Not applicable

CVL indicates central venous line; NA, not applicable; ETS, endotracheal suction; URI, upper respiratory infection; NP, nasopharyngeal culture; and BAL, bronchoalveolar lung lavage.
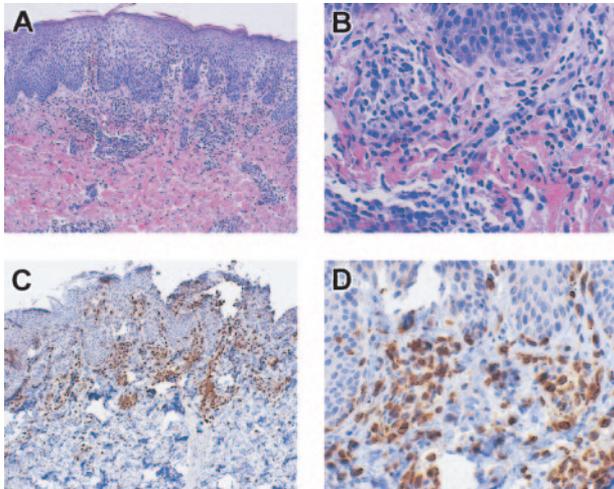
Figure 7. Skin biopsy from patient DIG009. Skin was stained with hematoxylin and eosin (original magnifications A, × 10; B, × 40) and was reacted with antibodies to CD3 (original magnifications C, × 10; D, × 40).

senescence of the small thymus gland. As there are few data points for TRECs, however, firm conclusions regarding thymic graft senescence cannot be drawn at this time. If thymic gland senescence does occur as is expected, it is hoped that the T cells that develop early on will maintain the repertoire as is seen in other transplantation settings.[30]

Evaluations of TCRBV repertoire showed normalization within one year (Figure 6). It is interesting to compare the immunoscope results in these DiGeorge patients after thymus transplantation to pediatric patients (not having DiGeorge syndrome) treated with cord blood transplantation.[27] The children treated with cord blood transplants did not normalize the immunoscope patterns until 3 years compared with one year in this study. Possibly the chemotherapy used for the cord blood transplants adversely affected thymic function resulting in a slower time course of normalization.

A number of factors relating to efficacy of thymic transplantation were examined retrospectively including HLA matching and length of culture. The patients with the most rapid development of T cells, DIG002 and DIG005, had closest HLA matching to the transplanted thymus. Other factors include time of culture and amount of thymus tissue transplanted per kilogram of patient weight. Ongoing studies will evaluate these factors in depth.

Rashes and autoimmune disease were seen after transplantation. Rashes,[21] cytopenias,[10,44] and thyroid disease[45] have been reported in complete DiGeorge patients prior to any therapy. After transplantation in the first 2 to 3 weeks, DIG009 and DIG010 developed oligoclonal host T cells associated with rash. These may represent extrathymically derived host T cells that have not been properly educated.[46,47] Alternatively, these may be host T cells that developed in the donor graft. It is possible that host antigen–presenting cells (APCs) had not yet migrated to the thymus to effect negative selection for host-reactive T cells. The resolution of the rashes may relate to the migration of the host APCs to the thymic graft. These hypotheses cannot be experimentally tested because of the risk to the patient that multiple biopsies would entail.

It is of interest to consider how thymic education might be working in these patients that received transplants. The thymus is involved in negative and positive selection. Negative selection eliminates those thymocytes that carry T-cell receptors that would react against self (see Starr et al[48] and Sprent and Kishimoto[49] for review). It is thought that APCs effect negative selection. Positive selection results in the development and proliferation of T cells carrying T-cell receptors that have moderate affinity to self–major histocompatibility complex (MHC) antigens. Positive selection is thought to occur on thymic epithelium (see Dhidgey and Boyd,[50] Res and Spits,[51] and Pawlowski et al[52] for review). The basis for positive and negative selection in these patients is speculation; however, it is difficult to experimentally prove what mechanisms are operative.

Donor and host APCs likely play a role in negative selection in this transplantation model. Donor APCs are transplanted because they are part of the graft. They may effect negative selection with respect to the donor. Host APCs migrate to the graft. They may effect negative selection with respect to host MHC thus preventing graft-versus-host disease. In a previous paper,[28] we reported the results for DIG001 for whom blood from the thymus donor was available for a mixed lymphocyte reaction. This patient was tolerant to the host but reactive against other donors.[28] This finding has been replicated in a patient with atypical complete DiGeorge syndrome for whom blood from the thymus donor was also available. That thymus transplant patient was enrolled in a different protocol (one for atypical patients that uses immunosuppression) than the one reported here (data not shown).

It is likely that positive selection for donor MHC occurs on the donor thymic epithelium. Possibly, positive selection for host may occur on host fibroblasts that are present at the surgical site and likely grow into the graft.[51-54] Positive selection has been seen on fibroblasts injected into murine thymuses.[53] When examining a patient for HLA restriction, one might expect to see some T cells restricted to host and others to donor. When experiments were done with DIG001 examining the response to tetanus toxoid by T-cell clones,[28] we only found host-restricted clones (data not shown). This may be secondary to the fact that all the cells in the peripheral lymph nodes are host. Thus, amplification after in vivo immunization may only occur in response to peptides presented by host MHC.

Acknowledgments

References

1. Thomas RA, Landing BH, Wells TR. Embryologic and other developmental considerations of thirty-eight possible variants of the DiGeorge Anomaly (DGA). Am J Med Genetics. 1987;3(suppl):43-66.

2. DiGeorge AM. Congenital absence of the thymus and its immunologic consequences: concurrence with congenital hypoparathyroidism. In: Bergsma D, ed. Birth Defects Original Article Series, IV [letter]. White Plains, NY: The National Foundation March of Dimes; 1968:116.

3. Conley ME, Beckwith JB, Mancer JF, Tenckhoff L. The spectrum of the DiGeorge syndrome. J Pediatr. 1979;94:883-890.

4. de la Chapelle A, Herva R, Koivisto M, Aula P. A deletion in chromosome 22 can cause DiGeorge syndrome. Hum Genet. 1981;57:253-256.

5. Driscoll DA, Salvin J, Sellinger B, et al. Prevalence of 22q11 microdeletions in DiGeorge and velocardiofacial syndromes: implications for genetic counseling and prenatal diagnosis. J Med Genet. 1993;30:813-817.

6. Hong R. The DiGeorge anomaly (CATCH 22, DiGeorge/velocardiofacial syndrome). Semin Hematol. 1998;35:282-290.

7. Muller W, Peter HH, Wilken M, et al. The DiGeorge Syndrome, I: clinical evaluation and course of partial and complete forms of the syndrome. Eur J Pediatr. 1988;147:496-502.

8. Wilson DI, Burn J, Scambler PJ, Goodship. Syndrome of the month: DiGeorge syndrome, part of CATCH-22. J Med Genet. 1993; 30:852-856.

9. Driscoll DA, Sullivan KE. DiGeorge syndrome: a chromosome 22q11.1 deletion syndrome. In: Ochs HD, Smith DIE, Puck JM, eds. Primary immunodeficiency diseases. New York, NY: Oxford University Press; 1999:476-489.

10. Jawad AF, McDonald-Mcginn DM, Zackai E, Sullivan KE. Immunologic features of chromosome 22q11.2 deletion syndrome (DiGeorge Syndrome/velocardiofacial syndrome). J Pediatr. 2001;139:715-723.

11. Metlay LA, Smythe PS, Miller ME. Familial CHARGE syndrome: clinical report with autopsy findings. Am J Med Genet. 1987;26:577-581.

12. Pagon RA, Graham Jr JM, Zonana J, Yong SL. Coloboma, congenital heart disease and choanal atresia with multiple anomalies: CHARGE association. J Pediatr. 1981;99:223-227.

13. Davenport SL, Hefner MA, Mitchell JA. The spectrum of clinical features in CHARGE syndrome. Clin Genet. 1986;29:298-310.

14. Muller W, Peter HH, Kallfelz HC, Franz A, Rieger CH. The DiGeorge sequence, II: immunologic findings in partial and complete forms of the disorder. Eur J Pediatr. 1989;149:96-103.

15. Bastian J, Law S, Vogler L, et al. Prediction of persistent immunodeficiency in the DiGeorge anomaly. J Pediatr. 1989;115:391-396.

16. Markert ML, Hummell DS, Rosenblatt HM, et al. Complete DiGeorge Syndrome: persistence of profound immunodeficiency. J Pediatr. 1998;132:15-21.

17. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LW. Control of lymphocyte recirculation in man, I: differential regulation of the peripheral lymph node homing receptor L-selection on T cells during the virgin to memory cell transition. J Immunol. 1993;150:1105-1121.

18. Livak F, Schatz D. T-cell receptor alpha locus V(D)J recombination by-products are abundant in thymocytes and mature T cells. Mol Cell Biol. 1996;16:609-618.

19. Kong F-k, Chen C-lH, Cooper MD. Thymic function can be accurately monitored by the level of

recent T cell emigrants in the circulation. Immunity. 1998;8:97-104.

20. Takeshita S, Toda M, Yamagishi H. Excision products of the T cell receptor gene support a progressive rearrangement model of the alpha/delta locus. EMBO J. 1989;8:3261-3270.

21. Archer E, Chuang T-Y, Hong R. Severe eczema in a patient with DiGeorge's syndrome. Cutis. 1990;45:455-459.

22. FDA Proposed Rule: Suitability determination for donors of human cellular and tissue-based products. Docket no. 97N–484S, CBER 9745 [FR Doc. 99-25378], pages 52696-52723. September 30, 1999. Written comments on information collection by November 1, 1999. Written comments by December 29, 1999.

23. Markert ML, Boeck A, Hale LP, et al. Thymus Transplantation in Complete DiGeorge Syndrome. N Engl J Med. 1999;341:1180-1189.

24. FDA Proposed Rule: Current good tissue practice for manufacturers of human cellular and tissue-based products; inspection and enforcement. Docket no. 97N–484P, CBER 9835 [FR Doc. 01-00447] , pages 1507-1559. January 8, 2001. Written comments by May 8, 2001. Written comments on information collection by February 7, 2001.

25. Hong R, Moore AL. Organ culture for thymus transplantation. Transplantation. 1996;61:444-448.

26. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature. 1998;396:690-695.

27. Klein AK, Patel DD, Gooding ME, et al. T-cell recovery in adults and children following umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2001;7:454-466.

28. Markert ML, Kostyu DD, Ward FE, et al. Successful formation of a chimeric human thymus allograft following transplantation of cultured postnatal human thymus. J Immunol. 1997;158:998-1005.

29. Markert ML, Watson TJ, Kaplan I, Hale LP, Haynes BF. The human thymic microenvironment during organ culture. Clin Immunol Immunopath. 1997;82:26-35.

30. Patel DD, Gooding ME, Parrott RE, Curtis KM, Haynes BF, Buckley RH. Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med. 2000;342:1325-1332.

31. Pannetier C, Levraud JP, Lim A, Even J, Kourilsky P. The immunoscope approach for the analysis of T-cell repertoires. In: Oksenberg J, ed. The Human Antigen T-cell Receptor: Selected protocols and applications. Austin, TX: RG Landes Company; 1997:287-325.

32. Pannetier C, Cochet M, Darche S, Casrouge A, Zoller M, Kourilsky P. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. Proc Natl Acad Sci U S A. 1993;90:4319-4323.

33. Bousso P, Wahn V, Douagi I, et al. Diversity, functionality, and stability of the T cell repertoire derived in vivo from a single human T cell precursor. Proc Natl Acad Sci U S A. 2000;97:274-278.

34. Daw SCM, Taylor C, Kraman M, et al. A common region of 10p deleted in DiGeorge and velocardiofacial syndromes. Nat Genet. 1996;13:458-460.

35. Erkeller-Yuksel FM, Deneys V, Yuksel B, et al. Age-related changes in human blood lymphocyte subpopulations. J Pediatr. 1992;120:216-222.

36. Markert ML, Mjure M, Harville TO, Hulka G, Old-

ham K. Severe laryngomalacia and bronchomalacia in DiGeorge syndrome and CHARGE association. Pediatr Pulmonol. 1997;24:364-369.

37. de Vries E, de Bruin-Versteeg S, Comans-Bitter WM, et al. Longitudinal survey of lymphocyte subpopulations in the first year of life. Pediatr Res. 2000;47:528-537.

38. Key G, Becker MH, Baron B, et al. New Ki-67-equivalent murine monoclonal antibodies (MIB 1-3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the Ki-67 epitope. Lab Invest. 1993;68:629-636.

39. Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med. 1999;340:508-516.

40. Goldsobel AB, Haas A, Stiehm ER. Bone marrow transplantation in DiGeorge syndrome. J Pediatr. 1987;111:40-44.

41. Borzy MS, Ridgway D, Noya FJ, Shearer WT. Successful bone marrow transplantation with split lymphoid chimerism in DiGeorge syndrome. J Clin Immunol. 1989;9:386-392.

42. Bowers DC, Lederman HM, Sicherer SH, Winkelstein JA, Chen AR. Immune reconstitution of complete DiGeorge anomaly by transplantation of unmobilized blood mononuclear cells. Lancet. 1998;352:1983-1984.

43. Bensoussan D, Le Deist F, Latger-Cannard V, et al. T-cell immune constitution after peripheral blood mononuclear cell transplantation in complete DiGeorge syndrome. Br J Haematol. 2002;117:899-906.

44. Duke SG, McGuirt WF Jr, Jewett T, Fasano MB. Velocardiofacial syndrome: incidence of immune cytopenias. Arch Otolaryngol Head Neck Surg. 2000;126:1141-1145.

45. Ham-Pong AJ, Cavallo A, Holman GH, Goldman AS. DiGeorge syndrome: long term survival complicated by Grave's disease. J Pediatr. 1985;106:619-620.

46. Davis CM, McLaughlin TM, Watson TJ, et al. Normalization of peripheral blood T cell receptor V beta repertoire after cultured postnatal human thymic transplantation in DiGeorge syndrome. J Clin Immunol. 1997;17:167-175.

47. Collard HR, Boeck A, McLaughlin TM, et al. Possible extrathymic development of nonfunctional T Cells in a patient with complete DiGeorge syndrome. Clin Immunol. 1999;91:156-162.

48. Starr TK, Jameson SC, Hogquist KA. Positive and negative T cell selection. Annu Rev Immunol. 2003;21:139-176.

49. Sprent J, Kishimoto H. The thymus and central tolerance. Philos Trans R Soc Lond B Biol Sci. 2001;356:609-616.

50. Dhidgey AP, Boyd RL. Thymic stromal cells and positive selection. APMIS. 2001;109:481-492.

51. Res R, Spits H. Developmental stages in the human thymus. Semin Immunol. 1999;11:39-46.

52. Pawlowski T, Elliot JD, Loh DY, Stearz UD. Positive selection of T lymphocytes on fibroblasts. Nature. 1993;364:642-645.

53. Hugo P, Kappler JW, McCormack JE, Marrack P. Fibroblasts can induce thymocyte positive selection in vivo. Proc Natl Acad Sci U S A. 1993;90:10335-10339.

54. Lilic M, Santori FR, Neilson EG, Frey AB, Vukmanovic S. The role of fibroblasts in thymocyte-positive selection. J Immunol. 2002;169:4945-4950.