

Complete DiGeorge syndrome: Development of rash, lymphadenopathy, and oligoclonal T cells in 5 cases

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Background: Five patients with DiGeorge syndrome presented with infections, skin rashes, and lymphadenopathy after the newborn period. T-cell counts and function varied greatly in each patient. Initial laboratory testing did not suggest athymia in these patients.

Objective: The purpose of this study was to determine whether the patients had significant immunodeficiency.

Methods: Research testing of peripheral blood included immunoscope evaluation of T-cell receptor β variable gene segment repertoire diversity, quantification of T-cell receptor rearrangement excision circles, and detection of naive T cells (expressing CD45RA and CD62L).

Results: The patients were classified as having DiGeorge syndrome on the basis of syndromic associations and heart, parathyroid, and immune abnormalities. Immunoscope evaluation revealed that the T-cell repertoires were strikingly oligoclonal in all patients. There were few recent thymic emigrants, as indicated by the very low numbers of naive T cells ($<50/\text{mm}^3$) and the absence of T-cell receptor rearrangement excision circles. These studies showed that all 5 patients were athymic. Two patients died, one from infection. No thymus was found during the complete autopsy performed on one patient.

Conclusion: Patients with DiGeorge syndrome, skin rash, and lymphadenopathy should undergo analysis of naive T-cell numbers and of T-cell receptor β variability segment repertoire to determine whether they are athymic, even if they have T cells with mitogen responsiveness. It is important for physicians to realize that patients with complete DiGeorge syndrome remain profoundly immunodeficient after development of these atypical features (rash, lymphadenopathy, and oligoclonal T cells). Prompt diagnosis is necessary for appropriate management. (*J Allergy Clin Immunol* 2004;113:734-41.)

Key words: T cells, DiGeorge syndrome, thymus, transplantation, immunodeficiency

DiGeorge syndrome is a congenital anomaly characterized by defects derived mainly from the third and fourth pharyngeal pouches, with additional abnormalities possibly extending from the first to sixth pharyngeal arch and first to fifth pharyngeal pouch.¹ Typically, the heart, the parathyroids, and the thymus are involved.¹⁻³ Other findings include gastroesophageal reflux, speech delay, laryngomalacia, cleft lip and palate, absent kidney, facial abnormalities, conductive or sensorineural deafness, malformed ears, seventh cranial nerve palsy, and hypothyroidism.^{2,3} Genetic and syndromic associations with DiGeorge syndrome include 22q11 hemizygosity,⁴ 10p13 hemizygosity,⁵ CHARGE association (coloboma, heart defect, choanal atresia, growth or development retardation, genital hypoplasia, ear anomaly or deafness),⁶⁻⁹ and diabetic embryopathy.^{10,11}

The spectrum of T-cell abnormalities in individuals with DiGeorge syndrome is quite broad. Individuals might have normal T-cell numbers and function, low T-cell numbers but fairly normal T-cell proliferative function,¹² or no T cells.¹³ The second group is referred to as having partial DiGeorge syndrome. The designation complete DiGeorge syndrome is reserved for the third group. Infants with complete DiGeorge syndrome have absence of thymic function in addition to other defects of the third and fourth pharyngeal pouches.^{12,13} These profoundly immunodeficient infants represent less than 1% of patients with DiGeorge syndrome.¹⁴

In the past, categorization of patients with DiGeorge syndrome as athymic required both profoundly low numbers of circulating T cells and very low T-cell proliferative responses to mitogens. The 5 patients described in this report show that athymia can be present in patients with DiGeorge Syndrome who have significant numbers of T cells that can respond to mitogens. We suggest that the criteria for athymia be refined on the basis of recent advances and include infants with very low levels of naive T cells ($<50/\text{mm}^3$ of CD3⁺ T cells coexpressing CD45RA and CD62L)¹⁵ and very low levels of T-cell receptor rearrangement excision circles (TRECs; <100 per 100,000 T cells).^{16,17} Using these criteria for athymia, we describe 5 patients with complete DiGeorge syndrome who presented with the classic findings of DiGeorge syndrome, namely heart and parathyroid defects, but who later had

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Abbreviations used

ALC:	Absolute lymphocyte count
CHARGE:	Coloboma, heart defect, choanal atresia, growth or developmental retardation, genital hypoplasia, ear defects including deafness
PCP:	<i>Pneumocystis carinii</i> pneumonia
RAG:	Recombinase activating gene
SCID:	Severe combined immunodeficiency
sjTREC:	Signal joint T-cell receptor rearrangement excision circle
TCRBV:	T-cell receptor β variable gene segment
TREC:	T-cell receptor rearrangement excision circle

rash, lymphadenopathy, and oligoclonal amplifications of T cells. These patients present a diagnostic challenge because they can be confused with patients with partial DiGeorge syndrome because of the presence of T cells. They are especially challenging if they are seen for the first time after development of the rash, lymphadenopathy, and oligoclonal T cells. Attention to the underlying third and fourth pharyngeal pouch defects and the oligoclonality of the T cells will lead to recognition of this atypical presentation of DiGeorge syndrome and allow appropriate therapy to be instituted.

METHODS

Patient participation

One of the authors (MLM) was initially contacted by referring physicians for treatment of patients diagnosed with DiGeorge syndrome (DIG101, DIG102, and DIG104) or for help in understanding a confusing immune phenotype (patient DIG106 at 8 months and patient DIG107 at 11 months). After their parents provided informed consent, all patients participated in immune studies according to a protocol approved by the Duke Institutional Review Board.

Cellular and genetic studies

Standard techniques were used for 3-color flow cytometry on anticoagulated blood samples.¹⁶ Naive CD4 and CD8 T cells were identified by gating on CD4 or CD8 and then analyzing these cells for coexpression of CD45RA and CD62L.¹⁵ Mitogen, antigen, and CD3 stimulations and mixed lymphocyte reactions were performed as previously described.¹⁸ Recombinase activating gene (RAG)—1 and RAG-2 sequencing was performed on PCR-amplified genomic DNA.

T-cell receptor rearrangement excision circle assay

The signal joint T-cell receptor rearrangement excision circle (sjTREC) assay^{16,17,19} was performed as previously described.^{17,20} In brief, sjTRECs in PBMCs or isolated CD3⁺ T cells were quantitated by using real-time quantitative-PCR with the 5'-nuclease assay with an ABI7700 system (Perkin-Elmer, Norwalk, Conn).²⁰ DNA was obtained from CD3⁺ T cells isolated from cryopreserved PBMCs by using MACS magnetic microbeads (Miltenyi-Biotech, Auburn, Calif). Real-time quantitative PCR was performed on lysate equivalent to 50,000 cells, with the following primers caccatcccttcaaccatgct and gccagctgcagggttttagg and the probe FAM-acacctctggtttgttaaaggtgccactTAMRA (MegaBases, Chicago, Ill). PCR reactions contained 0.5 U of Platinum Taq polymerase (Gibco, Grand Island, NY), 3.5 mmol/L MgCl₂, 0.2 mmol/L deoxyribonucleoside triphosphates, 500 nmol/L of each primer, 150 nmol/L probe, and Blue-636 reference dye (MegaBases).

Amplification conditions were 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds and 60°C for 1 minute. Samples were analyzed in duplicate and corrected to reflect 100,000 input cells. The sjTREC values were calculated by using a standard curve of T-cell receptor δ sjTREC plasmid DNA (1×10^7 to 1×10^2 molecules). The lower limit of detection in the assay was 100 molecules of sjTREC.

Immunohistochemistry

The evaluation of skin and lymph node biopsy specimens was performed as previously described.^{16,18} Antibodies used included CD3 (for frozen, mAb UCHT1 [Sigma Chemical Co, St Louis, Mo]; for paraffin, polyclonal rabbit anti-human CD3 [Dako, Carpinteria, Calif]), CD1a (mAb 010; Immunotech, Marseille, France), CD4 (for frozen, mAb Q4120 [Sigma]; for paraffin, mAb 1F6 [Novacastra Laboratories, Ltd, Newcastle upon Tyne, United Kingdom]), CD8 (for frozen, mAb UCHT-4 [Sigma]; for paraffin, mAb C8/144B [Dako]), TIA-1 (cytotoxic granules; mAb 2G9 [Immunotech]),²¹ Ki-67 (nuclear proliferation marker, mAb mib-1 [Immunotech]),²² cytokeratin (mAb AE1/AE3 cocktail [Boehringer Mannheim, Indianapolis, Ind]), and S100 (rabbit anti-cow polyclonal antibody [Dako]).

Immunoscope

The PCR-based immunoscopy technique was used as described to evaluate the T-cell receptor β variable gene segment (TCRBV) repertoire.^{18,23} Briefly, RNA was isolated from PBMCs and reverse transcribed into cDNA. PCR was used to amplify cDNAs containing each of 23 TCRBV families by using unique forward primers in the variable region and a reverse β constant primer. Products were visualized by using a fluorescently tagged internal reverse β constant primer. Profiles were characterized as oligoclonal if they contained 4 or fewer peaks, as polyclonal skewed if they had more than 4 peaks but were not Gaussian-like, and as polyclonal Gaussian if they had more than 4 peaks in a Gaussian-like distribution with the tallest peak centered at 10 amino acids. In each patient's profile in Fig 1, the lower right panel is the Jurkat control that has 2 peaks. Panels with no peaks represent technical problems.

RESULTS

Case reports

DIG101. Patient DIG101 was given a diagnosis early in life of complete DiGeorge syndrome on the basis of his 22q11 deletion, hypocalcemia, and T-cell lymphopenia (Tables I and II). He was transferred to Duke at 10 months of age with severe failure to thrive (weight of 3.44 kg vs birth weight of 3.92 kg), recurrent infections, eczematoid rash, and lymphadenopathy. Initially, the T-cell count was very low. It increased to a normal level with predominantly CD4 cells by 3 months of age but converted to predominantly double-negative cells by 8 months (Table II and Fig 2, A). A skin biopsy at 11 months (Fig 3) revealed that spongiotic dermatitis was present, with superficial and deep perivascular dermatitis and thickened epidermis. A marked CD3⁺ T-cell infiltrate was present at the dermal-epidermal interface, with T cells infiltrating the epidermis. T-cell clusters also surrounded eccrine glands.

While undergoing treatment for infections, the peripheral blood T-cell count increased to 9210/mm³ (Fig 2, A), and the response to PHA stimulation increased to 97,142 cpm (normal mean, 174,284 cpm with 1 SD; 59,813–507,830 cpm). The T cells were shown to be oligoclonal by means of flow cytometry. There were no naive T cells.

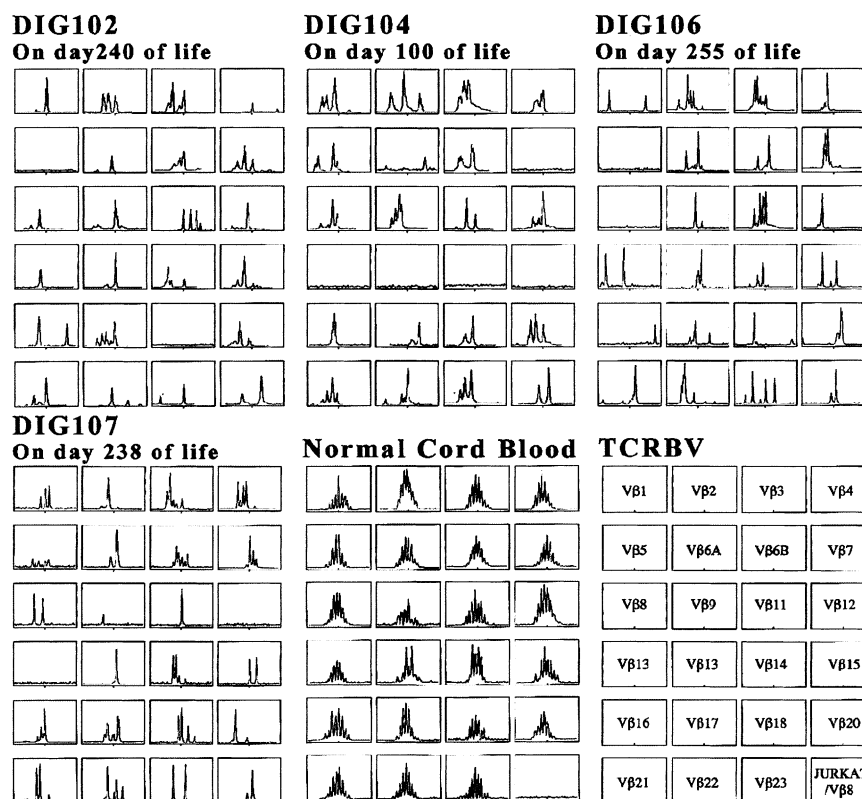


FIG 1. Immunoscopic evaluation of patients with atypical complete DiGeorge syndrome. The TCRBV family tested in each profile is indicated by the labels at the bottom right.

The CD3 T cells in general and the double-negative T-cell subset were both 90% TCR $\alpha\beta$ and 10% TCR $\gamma\delta$. An attempt was made to suppress the abnormal T cells with deoxycytosine (on days 338 and 344). Thymus tissue was transplanted on day 354; the patient died 5 weeks later of continuing infection and respiratory failure.

DIG102. Patient DIG102 presented at approximately 6 months of life with 25 T cells/mm³ (Fig 2, B), a PHA response of approximately 5500 cpm, and *Pneumocystis carinii* pneumonia (PCP). The diagnosis of DiGeorge syndrome was made by the pediatrician on the basis of the 22q11 deletion and T-cell lymphopenia (Tables I and II). When the PCP was treated, the T-cell count increased to 430/mm³, approximately half of which were double negative for CD4 and CD8. The total CD3 T cells and these double-negative T cells were both approximately half TCR $\alpha\beta$ and half TCR $\gamma\delta$. This increase in T-cell numbers was associated with an increase in PHA responsiveness, a strikingly oligoclonal immunoscopic evaluation of T-cell receptor repertoire (90% of the TCRBV families showing an oligoclonal profile) and absence of both naive T cells and TRECs (Table II). The patient had no proliferative response to tetanus toxoid, despite having received 3 immunizations. A skin biopsy specimen obtained on day 230 of life showed sparse spongiotic dermatitis. The inflammatory infiltrate was composed primarily of Langerhans cells, with a minority population of CD3⁺ T cells. A lymph node biopsy

specimen obtained on day 258 revealed marked T-cell depletion and histiocytic proliferation. A striking abnormality in this lymph node was the association of B-cell clusters with vessels and the absence of normal follicles. The histopathology of this lymph node closely resembled that of the lymph node from patient DIG106 (see below).

DIG104. Patient DIG104 was given a diagnosis of DiGeorge syndrome on the basis of hypocalcemia, athymia on chest magnetic resonance imaging, and T-cell lymphopenia (Tables I and II). Although the total CD3 count was 16/mm³ at 4 weeks, the T-cell count subsequently increased dramatically (Fig 2, C, and Table II). On day 37, the PHA response was reported by the cytogenetics laboratory as very poor (only one metaphase obtained). By 100 days, the PHA (Table II) and mixed lymphocyte reaction (data not shown) had increased significantly. Patient DIG104 had no TRECs on day 107 and less than 50 naive T cells per cubic millimeter. On day 101, 67% of the TCRBV families were determined to be oligoclonal by means of immunoscopy (Fig 1).

At 2 months of age, a rash developed that responded to tacrolimus after failure of topical steroids. A skin biopsy specimen obtained on day 113 after prolonged treatment with tacrolimus showed spongiotic dermatitis with CD3⁺ T-cell infiltrates within the dermis and scattered CD3 cells within the epidermis.

The patient had respiratory syncytial virus infection on day 113, received experimental thymus transplantation on

TABLE I. Patient characteristics

Patient ID no., sex, race-ethnicity	Syndromic association	Heart	Hypocalcemia*	Rash, lymphadenopathy, and other findings
DIG101, male, African American—Hispanic	22q11 Hemizygous	No defect	Yes	Rash (biopsy day 326), lymphadenopathy, severe FTT, infections with enterococcus, pseudomonas, methicillin-resistant <i>Staphylococcus aureus</i> and <i>Canadida</i> species, EBV PCR not performed
DIG102, male, white—non-Hispanic	22q11 Hemizygous, VCFS	No defect	Yes	Rash (biopsy day 230), lymphadenopathy (biopsy day 258), FTT, feeding intolerance, PCP right inguinal hernia, <i>Canadida albicans</i> in draining ear, adenovirus in stool, 0.1 copies EBV/PCR on blood (normal, 0-5, with >50 in active infection) day 370
DIG104, male, African American—non-Hispanic	22q11 Normal, 10p13 normal,† RAG1-RAG2 normal, diabetic mother	Patent foramen ovale	Yes	Polyhydramnios, vertebral anomalies at T7 and T8, hypocalcemic seizure on day 9 of life, no thymus on magnetic resonance imaging from the neck through the mediastinum (day 45), rash (biopsy day 113), lymphadenopathy, slightly abnormal BAER in left ear, GER, FFT, RSV infection on admission, 0.1 copies EBV/PCR on blood (normal, 0-5, with >50 in active infection) day 114
DIG106, male, white-Hispanic	22q11 Normal, 10p13 normal, RAG1-RAG2 normal, VCFS	VSD, large PDA, hypoplastic aortic arch with coarctation and sub AS	Yes	Cleft of soft palate and notch of the upper lip, malformed right ear, rash, lymphadenopathy (biopsy day 244), FTT, severe developmental delay, GER with Nissen and gastrostomy, ischemic brain injury, tracheostomy, right choanal atresia, EBV not detected by means of PCR on blood (day 317)
DIG107, male, white—non-Hispanic	22q11 Normal, 10p13 normal, RAG1-RAG2 normal, CHARGE	Small ASD	Yes	Poor feeding, swallowing dysfunction, GER, left choanal atresia, abnormal BAER on left, FTT, intermittent rash, adenopathy (biopsy day 161), seventh nerve palsy, multiple aspiration pneumonias, vocal cord paralysis, EBV not detected by means of PCR on blood (day 379)

FTT, Failure to thrive; VCFS, velocardiofacial syndrome; BAER, brainstem auditory evoked response; GER, gastroesophageal reflux; VSD, ventricular septal defect; PDA, patent ductus arteriosus; AS, aortic stenosis, ASD, atrioseptal defect.
*Hypocalcemia in these infants was manifested by a requirement of supplemental calcium to keep the ionized blood calcium level at greater than 1.0 mmol/L.
†10p13 Hemizygosity was assessed by means of high-resolution chromosome analysis. All patients to date are reported to have 10p13 hemizygosity show a deletion of chromosome bandings (Scrambler P, personal communication).

day 119, and died of respiratory syncytial virus infection on day 256. At autopsy, the patient was athymic. The lymph nodes identified had moderate to severe lymphodepletion, although cortex and medulla could be identified. Some lymph nodes lacked B-cell follicles, and others contained only rudimentary B-cell follicles lacking germinal centers.

DIG106. Patient DIG106 had multiple anomalies and was given a diagnosis of velocardiofacial syndrome on the basis of heart and facial findings (Table I). Although the initial absolute lymphocyte count (ALC) was 1346/mm³, all other ALCs in the first 3 months of life were less than 1000/mm³ (Fig 2, D). Test results for 22q11 were normal on day 26; however, the cytogenetics laboratory reported very few metaphases, suggesting minimal T-cell proliferative response to PHA. At 7 months, patient DIG106

presented to the hospital with fever, massive lymphadenopathy, and leukocytosis of 38,000/mm³ (absolute neutrophil count of 9216/mm³, ALC of 12,672/mm³, and absolute eosinophil count of 11,904/mm³). One month later, with an ALC of 33,801/mm³, the patient underwent left axillary lymph node biopsy. Clusters of CD20⁺ B lymphocytes lacking classic germinal centers were surrounded by pale-staining T cells and histiocytes. The majority of the CD3⁺ cells were CD8⁺ cells. The patient was treated with steroids. T cells were first evaluated on day 255. Sixty-one percent of the lymphocytes were CD8⁺ T cells. There were no naive T cells and no TRECs. The immunoscopy study from day 255 (Fig 1) found that 65% of the TCRBV families were oligoclonal. A skin biopsy specimen showed that small numbers of T cells were present in the epidermis, with

TABLE II. Laboratory data

Patient ID no.	Initial T-cell no. and age	Highest T-cell no. and age	Naive T-cell no. and age	Initial PHA response (ctrl)* and age	Highest PHA (ctrl) and age	CD3 TRECs and age	Genetic origin of T cells and age
DIG101	266/mm ³ , 72 d†	24,146/mm ³ , 379 d‡	21/mm ³ , 256 d	5115 (89,901) cpm, 256 d	97,142 (166,406) cpm, 330 d§	Not done	Host by 22q11 hemizygosity done on PBMC by interphase FISH, day 75 of life; all host by FISH XY, day 295
DIG102	24/mm ³ , 217 d	430/mm ³ , 248 d	Not detectable, 217 d	5615 (154,264) cpm, 205 d	65,397 (141,866) cpm, 248 d§	0 per 100,000 T cells; 253 d	Host by 22q11 hemizygosity, day 204; host by RFLP, day 238; day 259 host by FISH XY in lymph node, day 259
DIG104	16/mm ³ , 31 d	7942/mm ³ , 107 d	29/mm ³ , 100 d	52,236 (157,333) cpm, 100 d	54,180 (277,680) cpm, 107 d§	0 per 100,000 T cells; 107 d	Host, day 113, by FISH for XY in isolated T cells
DIG106	2113/mm ³ , 255 d	7684/mm ³ , 316 d	14/mm ³ , 255 d	4869 (275,946) cpm, 255 d	40,526 (243,853) cpm, 316 d	0 per 100,000 T cells; 255 d	Host by RFLP, day 374 in isolated T cells
DIG107	1524/mm ³ , 158 d	1524/mm ³ , 158 d	0/mm ³ , 338 d	553 cpm (not available)¶, 305 d	21,326 (231,258) cpm, 318 d	Not done	Host by FISH for XY in lymph node cells, day 161

ctrl, Control; FISH, fluorescent in situ hybridization.

*Ctrl refers to the proliferation of a normal control run in a parallel with the patient sample. Note: backgrounds (proliferation of cells plus medium without mitogen) were all less than 600 cpm.

†Data from referring physician before patient being referred to MLM.

‡DIG101 underwent thymus transplantation on day 354 after receiving deoxycoryformycin twice on days 338 and 344 as immunosuppression. The deoxycoryformycin did not suppress the T cells that were 3768/mm³ shortly before thymus transplantation. At autopsy, the graft appeared to be rejected. Because the graft did not function, the T-cell count from day 379 (after transplantation) is included.

§PHA proliferative responses of more than 50,000 cpm were noted twice in patients DIG101, DIG102, and DIG104. They only decreased after immunosuppressive therapy in patients DIG101 and DIG104. The response to PHA decreased spontaneously to 39,824 cpm in patient DIG102 who was then immunosuppressed.

||In clinical testing at this institution, interphase FISH done on 200 cells has a sensitivity of 0.5%. RFLP has a sensitivity of 1%.

¶The initial study was done in an outside laboratory, and the controls are not available. Nine days later at Duke, the PHA response was 21,326 cpm, with a control of 231,258 cpm.

larger numbers surrounding vessels in the dermis or just scattered in the dermis.

DIG107. Patient DIG107 had multiple congenital anomalies (Table I), with failure to thrive and intermittent dermatitis of the body and scalp for the first 8 months of life. A lymph node biopsy specimen obtained at 5 months because of lymphadenopathy was described as showing dermatopathic lymphadenopathy and showed no classic follicles but some residual germinal centers. A cytogenetics report at this time stated that no mitoses were observed on a peripheral blood specimen processed for chromosome analysis (by using stimulation with PHA). The serum IgE level at age 6 months was 4270 IU/mL (normal, 0-74 IU/mL; Fig 2, G). T cells were decreased but not absent (Table II and Fig 2, E). By 10 months of age, the rash and lymphadenopathy spontaneously resolved. The patient had PCP at 10 months and was started on steroids. The T-cell numbers were markedly reduced from the previous examination. The mitogen responses were at background (with the patient taking steroids); there were no naive T cells. The immunoscope study at day 338 (Fig 1) showed that 76% of the TCRBV families were oligoclonal. A skin biopsy specimen taken at 1 year of age showed a similar pattern as was observed in patient DIG106, with T-cell infiltrates around the epithelium of hair follicles and scattered and surrounding vessels in the dermis. At this time (1 year), the diagnosis of CHARGE was made on the basis of the patient's heart defect, choanal atresia, growth retardation, ear phenotype, and deafness. The diagnosis of DiGeorge syndrome was also made on

the basis of the hypocalcemia, heart defect, lack of naive T cells, and relationship with CHARGE association.

In summary, all 5 infants had rash and lymphadenopathy associated with oligoclonal proliferations of host T cells at different times after birth. There was no evidence for EBV causing these proliferations (Table I). TCRγδ T cells were 1% to 2% of the total T cells in patients DIG104, DIG106, and DIG107; 10% of the total T cells in patient DIG101; and one third to one half of the T cells in patient DIG102. Oligoclonality was demonstrated on the basis of immunoscope profiles on all patients except for patient DIG101 (Fig 1), who presented before this technology was available. In most patients, these oligoclonal T cells proliferated at low to moderate levels in response to mitogens (Table II). Importantly, the oligoclonal T cells showed no markers of recent thymic emigrants (Table II). Specifically, there were fewer than 50/mm³ T cells coexpressing CD45RA and CD62L and fewer than 100 TRECs per 100,000 T cells. The absence of markers for recent thymic emigrants was supported in all patients by the absence of thymus on chest radiographs.

DISCUSSION

All 5 patients had DiGeorge syndrome. Despite not having heart defects, patients DIG101 and DIG102 were hemizygous for 22q11 and had hypocalcemia. Patient DIG106 had a typical heart lesion for DiGeorge syndrome and also had hypocalcemia. Both patient DIG104 and

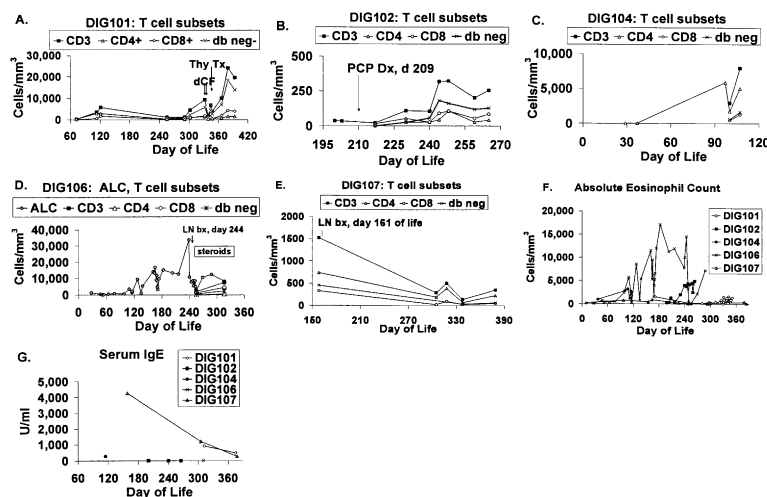


FIG 2. Immune studies in patients with atypical complete DiGeorge syndrome: **A**, patient DIG101; **B**, patient DIG102; **C**, patient DIG104; **D**, patient DIG106; **E**, patient DIG107; **F**, absolute eosinophil counts; **G**, serum IgE levels. The 25th to 75th percentiles values for infants follow²⁴: ALC, 2700-5400/mm³; CD3, 1700-3600/mm³; CD4, 1700-2800/mm³; CD8, 800-1200/mm³; absolute eosinophil count, less than 350/mm³; IgE, 0-74 U/mL.

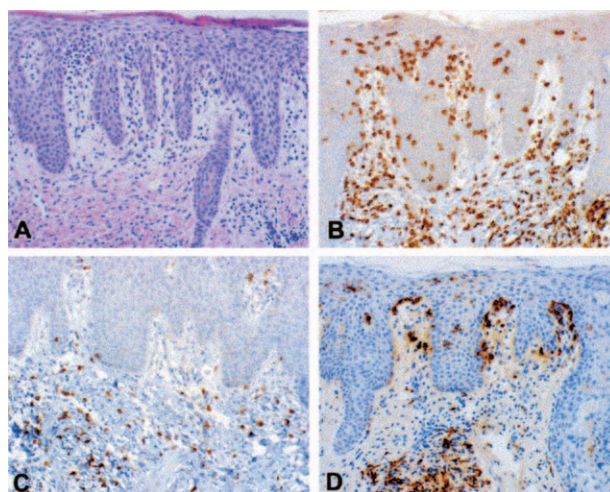


FIG 3. Skin biopsy specimen of patient DIG101 on day 326 of life: **A**, hematoxylin and eosin, original magnification 20 $\times$; **B**, CD3, original magnification 20 $\times$; **C**, CD8, original magnification 20 $\times$; **D**, S100, original magnification 20 $\times$. Note the large numbers of T cells in the dermis.

patient DIG107 had profound hypocalcemia, implicating both the third and fourth pharyngeal pouches. Patient DIG107 also had a heart defect and the associated pharyngeal arch defects of seventh nerve palsy (second arch) and choanal atresia (first arch). For the patients who were normal at 22q11, chromosome analysis was used to rule out 10p13 hemizygosity, which has been seen in a small number of children with DiGeorge syndrome and slightly low T-cell numbers.⁵ RAG-1 and RAG-2 sequencing was used to rule out Omenn syndrome.²⁵⁻²⁸ The syndromic associations in this group of patients (Table I) are similar to those of previously reported patients with complete DiGeorge syndrome.¹⁸

All patients were athymic. In patients DIG101, DIG102, and DIG104 the initial T-cell counts were very low, supporting the diagnosis of athymia. In patient

DIG104, in addition, an autopsy confirmed the absence of thymus. One can infer a very low total T-cell count in patient DIG106 because of the profoundly low ALCs early in life (124/mm³ on day 51) and the low mitotic index on day 26. The T cells that developed by 8.5 months, when they were first tested, lacked markers of thymic development, namely coexpression of CD45RA and CD62L and TRECs. Patient DIG107 did not undergo T-cell testing until 5 months of age, but his initial ALC at 9 days was very low at 954/mm³. The first testing for naive T cells in patient DIG107 was performed at 11 months. The patient had 10/mm³ naive T cells. We propose that athymia be defined as the presence of less than 50/mm³ naive T cells and less than 100 TRECs/100,000 T cells (if there are enough T cells for this test to be done). By these criteria, the 5 patients were athymic.

These 5 patients are distinguished from others with complete DiGeorge syndrome by their skin rash with infiltrating T cells, lymphadenopathy, and oligoclonal T cells, which proliferated in response to PHA. The patients developed the rash, lymphadenopathy, and oligoclonal T cells in a stochastic fashion. All patients had oligoclonal expansions in more than 50% of TCRBV families. Both patient DIG104 and patient DIG101 had these atypical features at approximately 3 months of age (Table I). These same atypical features developed later in the other patients, with patient DIG102 undergoing lymph node biopsy at 8 months, patient DIG106 at 8 months, and patient DIG107 at 5 months.

Patients with DiGeorge syndrome with rash have been described in the past.^{25,29-32} However, in those cases the T cells did not respond to mitogens, and thus it was clear that the patients were severely immunodeficient. The rash and lymphadenopathy that develop late in patients with complete DiGeorge syndrome are similar to the findings in Omenn syndrome,²⁵⁻²⁸ in maternal engraftment in infants with severe combined immunodeficiency (SCID),³³⁻³⁵ and in maternal engraftment in infants with DiGeorge syndrome.³⁶ They all have rash, eosinophilia, increased IgE levels, diarrhea, and lymphadenopathy. Although the patients' clinical findings were similar to those of patients with immunodeficient SCID or patients with DiGeorge syndrome with maternal engraftment, the patients could be distinguished because their T cells were all genetically host (Table II). Most important for distinguishing the atypical form of DiGeorge syndrome from Omenn syndrome or SCID is the presence of the other pharyngeal pouch defects, such as hypoparathyroidism, which is characteristic of DiGeorge syndrome. Genetic testing is useful if the patient is 22q11 or 10p13 hemizygous; the former is present in less than half of patients with complete DiGeorge syndrome,¹⁸ and the latter is very rare.⁵

The presence of T cells that proliferated in response to PHA made the diagnosis of athymia difficult for the consultants who were asked to evaluate patients DIG102, DIG106, and DIG107. In the case of patient DIG102, the presence of T cells with PHA responsiveness raised the possibility that this patient had partial DiGeorge syndrome and was spontaneously recovering. For patient DIG106, DiGeorge syndrome was not recognized while he was hospitalized during the lymphopenic phase or when he was an outpatient at the time that his ALC increased. The rash, lymphadenopathy, and lymphocytosis were confusing in the absence of the diagnosis of DiGeorge syndrome.

In patient DIG107 the CHARGE diagnosis was initially missed, as was the diagnosis of DiGeorge syndrome, as detailed above. Patient DIG107 had changed physicians because of family relocation, and the immune findings appeared to be contradictory. The very low mitotic index on chromosome testing in patients DIG106 and DIG107 should have raised the possibility of a T-cell deficiency. A test of T-cell proliferative responses to PHA at that time might have led to the appropriate diagnosis 7 months earlier in both patients. This highlights the importance of

communication from the cytogenetics laboratory to the physicians to inform the latter, especially when 22q11 testing has been ordered, that a low mitotic index might reflect poor T-cell function and that T-cell studies are indicated. In addition, the cytogenetics report should state that 22q11 hemizygosity is not required for the diagnosis of DiGeorge syndrome.

The authors were aided in the diagnosis of complete DiGeorge syndrome in these patients by using the striking clinical phenotype of rash and lymphadenopathy associated with oligoclonal T cells in patient DIG101, who clearly had been born with complete DiGeorge syndrome. Despite this, the authors studied patient DIG102 for 2 months before ruling out partial DiGeorge syndrome and concluding that he was athymic. After seeing the similarities in these 2 patients, the pattern in patients DIG104, DIG106, and DIG107 was quickly identified as associated with complete, and not partial, DiGeorge syndrome.

Some immunologists believe that the emphasis on partial versus complete DiGeorge syndrome is a matter of semantics and that care should be dictated by the level of immunodeficiency based on lymphocyte proliferative responses to mitogens and antigens. As can be seen by these cases, an increase in proliferative function to mitogens should not be interpreted as the spontaneous improvement seen in many patients with partial DiGeorge syndrome. The pediatrician must recognize DiGeorge syndrome based on defects of the heart, parathyroid, or both and history of lymphopenia or absence of T cells. The rash and lymphadenopathy should raise the possibility that the child has progressed to an atypical form of complete DiGeorge syndrome. The infant should be referred to a center that can perform flow cytometry, proliferative stimulations, and research testing to measure TRECs or quantitate naive T-cell numbers by means of flow cytometry or immunoscopy. The oligoclonal TCRBV repertoire gives the clue that there is a thymic problem. The lack of naive T-cell markers (CD45RA and CD62L coexpression and TRECs) should be used to quickly make the diagnosis.

In summary, we describe a group of patients with complete DiGeorge syndrome, who, despite having T cells that respond to mitogens, have no evidence of thymic function. Although they can have high T-cell numbers, these patients have profound immunodeficiency, with its associated clinical findings. The diagnosis of athymia in these patients requires specialized testing, such as repertoire analysis by means of immunoscopy or flow cytometry, TREC analysis, and/or the use of specialized markers to detect naive T cells by means of flow cytometry. This report underscores the need to assess T-cell clonality and to quantitate naive T-cell numbers in infants presenting with immune deficiency to manage their care appropriately. We have treated the patients in this report with thymus transplantation after pretransplantation immunosuppression. The results are encouraging and are reported elsewhere.³⁷ Because this therapy is being developed to correct the immunodeficiency in complete DiGeorge syndrome, it is critical that an accurate diagnosis of

athymia in such patients is established to ensure access to appropriate therapy.

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