Published in final edited form as:

Immunol Res. 2009 ; 44(1-3): 61–70. doi:10.1007/s12026-008-8082-5.

Thymus Transplantation in Complete DiGeorge Anomaly

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Abstract

Complete DiGeorge anomaly is characterized by athymia, congenital heart disease and hypoparathyroidism. This congenital disease is fatal by age 2 years unless immune reconstitution is successful. There are multiple underlying syndromes associated with complete DiGeorge anomaly including 22q11 hemizygosity in approximately 50%, CHARGE association in approximately 25%, and diabetic embryopathy in approximately 15%. Approximately one third of patients present with rash and lymphadenopathy associated with oligoclonal “host” T cells. This condition resembles Omenn syndrome. Immunosuppression is necessary to control the oligoclonal T cells. The results of thymus transplantation are reported for a series of 50 patients, 36 of whom survive. The survivors develop naïve T cells and a diverse T cell repertoire.

Keywords

primary immunodeficiency; DiGeorge anomaly; thymus transplantation

Introduction

DiGeorge anomaly is a congenital malformation with multiple etiologies. The characteristic findings include thymus hypoplasia or aplasia, congenital heart disease, and hypoparathyroidism.[1, 2] Total absence of the thymus occurs in approximately 1% of patients. These patients are said to have complete DiGeorge anomaly. Because they are athymic, patients with “complete” DiGeorge anomaly do not develop educated T cells, hence they are at risk of death from infection. Approximately two thirds of patients die by one year of age and the remainder die by 2 years of age. The vast majority of infants with DiGeorge anomaly have “partial” DiGeorge anomaly. Although their T cell numbers are low, these patients do not need immune restorative therapy.[3]

Often overlooked is the diversity of etiologies leading to DiGeorge anomaly. The term “anomaly” implies the presence of more than one etiology in contrast to the term “syndrome” which implies one etiology. In a series of 50 infants with complete DiGeorge anomaly, approximately half were found to be hemizygous for 22q11 [4], approximately one quarter had CHARGE association (coloboma, heart defect, choanal atresia, growth or mental

retardation, genital hypoplasia, and ear anomalies or deafness)[5, 6], and approximately 15% were infants of diabetic mothers.[7–9]

In addition to the diversity of underlying genetic or syndromic associations, there is diversity in the clinical presentation of complete DiGeorge anomaly. The heart and parathyroid gland may not be affected or may be mildly affected. Approximately 80% of infants with complete DiGeorge anomaly require calcium and/or calcitriol supplementation because of hypoparathyroidism.[7] Although a majority will require heart surgery, the prevalence of conotruncal heart defects is less than 50%.[7] In complete DiGeorge anomaly patients without 22q11 hemizygosity, fewer than 20% have conotruncal heart defects.[7]

There are two phenotypes associated with complete DiGeorge anomaly. In approximately two thirds of patients, there are few T cells and no rash or lymphadenopathy. This is called typical complete DiGeorge anomaly. In the remaining third of patients, a rash and lymphadenopathy develop associated with oligoclonal “host” (not maternal) T cells.[10] This is called “atypical” complete DiGeorge anomaly. It clinically resembles Omenn syndrome. The oligoclonal T cells are controlled with immunosuppression.

The diagnosis of athymia is made by flow cytometry using antibodies reactive with T cells (CD3, CD4, CD8) and recent thymic emigrants. Recent thymic emigrants, also known as naïve T cells, co-express CD45RA and CD62L. Infants with typical complete DiGeorge anomaly usually have fewer than 50/mm³ T cells. However, if higher numbers of circulating T cells are present, the diagnosis of complete DiGeorge anomaly requires the naïve T cells to be less than 50/mm³ or less than 5% of the total T cells.[7] The T cells associated with atypical complete DiGeorge anomaly may be single positive CD4+ T cells, single positive CD8+ T cells, double negative (CD4–CD8–) T cells or a combination of those cells. They express T cell receptor (TCR) $\alpha\beta$ chains. The T cells are oligoclonal based on spectratyping or flow cytometry analysis of T cell receptor variable beta (TCRBV) chain composition (Figure 1).[7, 10] These T cells often bear activation markers such as HLA-DR, CD25, and CD71 (Figure 2). Although the proliferative T cell response to the mitogen phytohemagglutinin (PHA) may be very low, in some patients it can be normal reflecting the aggressive nature of these cells.

Before making the diagnosis of atypical complete DiGeorge syndrome, one must rule out maternal engraftment. This is done by comparing DNA from T cells isolated from the patient with DNA from the patient’s buccal swab and DNA from the mother. Alternatively if the patient is male, the isolated T cells can be tested for XX versus XY by fluorescent *in situ* hybridization.

The rash in atypical complete DiGeorge anomaly often resembles atopic dermatitis [10], however, it can also be predominantly flaky or can progress to the appearance of severe Omenn’s syndrome (Figure 3). The rash is pruritic. The pathologic findings are usually spongiotic dermatitis with lymphocytes.[11] In addition to infiltrating the skin, these T cells can infiltrate the liver leading to elevated transaminases (Figure 4) and can also lead to the pathologic appearance of graft versus host disease in the gut.

Findings characteristic of DiGeorge anomaly lead to the correct diagnosis of athymia instead of a bone marrow defect such as Rag1 or Rag2 deficiency or atopic dermatitis. In particular, any of the following findings would lead one to the diagnosis of complete DiGeorge anomaly: cleft lip/palate, coloboma, 7th nerve palsy, butterfly vertebrae, single kidney, heart defect, hypoparathyroidism, abnormal ears or hearing deficit, choanal atresia, and genital hypoplasia.

The clinical care of patients with complete DiGeorge anomaly is challenging. Approximately a third have respiratory concerns such as laryngomalacia or aspiration that may lead to tracheostomy. Others may need ventilator support because of congenital chest wall/rib anomalies or congenital heart disease. Another third of patients present with findings such as heart failure, severe infections, profound malnutrition, or graft versus host disease from unirradiated blood transfusions. The hypoparathyroidism leads to low calcium values and, with overzealous replacement, nephrocalcinosis and kidney stones. Lastly, the patients with atypical complete DiGeorge anomaly require immunosuppression with medications such as steroids and cyclosporine which may lead to hypertension and renal complications.

Presentation occurs early in life. In the author's series of infants transplanted with thymus tissue, approximately one third were transplanted between 2 and 4 months. The median age at transplantation was 4.3 months.

Thymus transplantation, methods

The author has performed transplantation of postnatal allogeneic thymus tissue into 50 infants with complete DiGeorge anomaly. The mechanism is thought to be migration of recipient bone marrow CD34+ stem cells from the bone marrow to the thymus tissue and development in that tissue into genetically recipient T cells.

Thymus tissue is procured and processed under an Investigational New Drug (IND) application with the FDA. The thymus is obtained as discarded tissue from infants under 9 months of age undergoing cardiac surgery. Any thymus that is removed in the course of the operation is placed in a sterile container and transported to the laboratory where it is sliced into 0.5 mm strips and held in culture. Consent is obtained from the parents for use of the tissue and for testing of the tissue for hepatitis B and C, and Epstein Barr virus and cytomegalovirus. The biologic mother is tested per Food and Drug Administration (FDA) guidelines for solid organ donation.[12] All infants over 1 month of age undergo the same testing as their mothers. During the 2–3 week culture period, the thymus is maintained using Good Tissue Practice.[13] The culture medium has been described.[14] Multiple cultures are obtained to assure sterility of the product.[14] Lot release criteria include immunohistochemistry to show viability of the epithelium and depletion of the thymocytes. [14]

HLA matching is not required for thymus transplantation. Approximately one third of transplants have no matching with the recipients. This does not appear to affect T cell counts or T cell function.[15]

The thymus tissue is transplanted in an open procedure in the operating room.[16] The slices of tissue are placed in individual pockets in the quadriceps muscles. Twenty to sixty slices are usually implanted. A suture closes each pocket and marks the site of each tissue implant. After 2–3 months, a biopsy is obtained in the operating room. The muscle under a suture is removed and examined by immunohistochemistry for evidence of thymopoiesis (Figure 5). [17] In particular, lacy cytokeratin associated with T cells bearing markers of cortical thymocytes (CD1a or Ki-67) is sought. If thymopoiesis is found, naïve T cells usually appear in the blood within 2–4 months.

Thymus transplantation, survival

Fifty infants with complete DiGeorge anomaly have been transplanted with thymus tissue by the author. The one year survival rate is 73% (35/48). See the Kaplan Meier curve (Figure 6). Of note, only one subject of the 35 surviving to 1 year has died after 1 year after transplantation. Most deaths occur prior to development of T cells. The etiology usually is infection. Of 21 viral infections documented in the first 6 months after transplantation, there were 4 deaths, 2 from respiratory syncytial virus and 2 from CMV. Other patients were very sick (e.g. on ventilators) during this period with these infections and others such as parainfluenza virus.

Thymus transplantation, immune results

T cells develop in 4–5 months after thymus transplantation in infants with typical complete DiGeorge anomaly. If suppression is needed as for infants with atypical complete DiGeorge anomaly, the T cell development usually occurs at 5–7 months. In atypical patients, the first signs of thymus function in the blood are change from $\gamma\delta$ to $\alpha\beta$ T cells and change from predominant CD8 single positive or double negative T cells to predominantly CD4 single positive T cells. Naïve T cells usually appear soon afterward. Figure 7 shows CD3, CD4, CD8, and naïve CD4 and CD8 counts from subjects given transplants without immunosuppression. Although the numbers of T cells remain at or slightly below the 10th percentile for age, the percentage of naïve T cells can be quite robust. CD4:CD8 ratios tend to be slightly high for age (not shown). Subjects treated with immunosuppression have similar outcomes.[7] The TCRBV repertoire normalizes.[18] After the TCRBV repertoire normalizes and naïve T cells appear, the immunosuppression used in atypical subjects can be weaned off without reappearance of the rash. These T cells are genetically host.

T cell function also normalizes after transplantation. Normal proliferative responses to the mitogen PHA develop within 9–12 months. The ability to mount proliferative responses to specific antigens such as tetanus toxoid develops at approximately one year.

B cell function has also been studied. Immunoglobulin replacement is stopped at 2 years after transplantation. IgG levels have remained in the normal range for most subjects (only 7%, 1 of 15, being low for age).[7] Approximately 10% (2 of 21) are low for IgA.[7] This was expected since IgG deficiency is associated with partial DiGeorge anomaly.[19] Approximately 20% of transplanted subjects have low IgM levels after 1 year after transplantation. With respect to antigen-specific antibodies, most infants respond to tetanus

toxoid vaccination with a normal antibody titer.[7] The infants make antibodies in response to immunization with unconjugated pneumococcal antigens but respond to fewer serotypes than normal children.[7, 20] Only a third of subjects have normal antibody responses to isohemagglutinins.[7]

Clinical course after thymus transplantation, adverse reactions

There have been very few adverse events associated with the transplantation procedure. A few subjects developed infections at the surgical site or a minor dehiscence of the skin. Of note there has been no graft versus host disease associated with thymus donor T cells.

Clinical course after thymus transplantation, infections

Clinically, a major finding after naïve T cells develop is that subjects no longer suffer life threatening complications from viral infections. After 6 months after transplantation, we have detected 7 episodes of RSV, 3 of parainfluenza virus, and 1 varicella infection. Many of these were detected because of hypocalcemia. (Low calcium levels usually are associated with infection so cultures are obtained at times of low calcium.) The infants who continue to require hospitalization for infections are those with bronchomalacia who have anatomic reasons for ongoing infections.

Clinical course after thymus transplantation, autoimmune disease

Autoimmune disease has been the most common adverse event. Five infants developed Hashimoto thyroiditis. Six additional subjects developed thyroid disease prior to transplantation. That finding and the 20% prevalence rate of thyroid disease in adults with partial DiGeorge anomaly [21] make the role of thymus transplantation unclear in thyroid disease. Cytopenias have been seen in the first year after transplantation.[7] All have resolved with intravenous immunoglobulin or steroid therapy. One subject developed nephrotic syndrome which resolved after steroid therapy. One subject (who had thyroid disease) also developed alopecia totalis.[7] One subject developed autoimmune hepatitis. The elevated transaminases normalized with immunosuppressive treatment. One atypical subject developed granulomas in the skin associated with rash approximately 2 months after transplantation. This may be a characteristic of atypical DiGeorge anomaly, however, as this has now been seen in another atypical subject prior to transplantation. Lastly, one subject had a life threatening enteritis/colitis characterized by denudation of the gut and infiltration of the gut with neutrophils and lymphocytes.[7] This was only controlled with high doses of immunosuppressive medication which led to a fungal infection and death. This subject received the highest dose of thymus given to date. A maximum dose has been set which hopefully will prevent any occurrences of this problem.

Acknowledgments

The technical assistance of Marilyn Alexieff, Jie Li, Chia-San Hsieh, Jennifer Lonon, Julie Cox, and Anita Croasmun is appreciated. The collaboration of surgeons Drs. Henry Rice, Jeffrey Hoehner, James Jaggars, and Andy Lodge is acknowledged. We are grateful for the clinical assistance of the faculty and fellows of the Duke Division of Pediatric Allergy and Immunology. Funding was from the National Institute of Health grants R01 AI 47040, R21 AI 60967, R01 AI 54843, and from the Food and Drug Administration Office of Orphan Products

Development, grant FD-R-2606. MLM is a member of the Duke Comprehensive Cancer Center of which the flow cytometry facility under Dr. Michael Cook was very helpful for this research.

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Figure 1.

Oligoclonal T cells found in atypical complete DiGeorge anomaly. Flow cytometry with antibodies reactive with members of T cell receptor beta chain variable families is shown. Family 5.1 represented 80% of the circulating CD4 T cells. The distribution of the patient is shown as filled symbols and that of the healthy adult control by open symbols.

Figure 2.

Activated phenotype of the oligoclonal T cells in atypical complete DiGeorge anomaly. Flow cytometry was used to examine CD3 T cells with antibody directed against the T cell activation markers HLA-DR and CD71. The left panel includes a healthy adult control; the right panel is a patient with atypical complete DiGeorge anomaly prior to thymus transplantation. Eighty six percent of the patient's CD3 T cells are activated expressing both markers.

Figure 3.
Rash in atypical complete DiGeorge anomaly. This patient had not yet received any immunosuppression.

Figure 4.

Liver involvement in atypical complete DiGeorge anomaly. A) Low power view of liver with portal triad and central vein. B) High power view of liver with mononuclear cells, granulomatous inflammation and fibrous expansion in the portal triad. C) High power view of T cells visualized by brown color on immunohistochemistry in the portal triad.

Figure 5. Thymus graft of a typical subject taken 75 days after thymus transplantation. A) CD1a, B) Ki-67, C) CD3, and D) cytokeratin reactivity. The T cells express CD1, characteristic of cortical thymocytes. The cytokeratin is lacey, a normal pattern.

Figure 6.

Kaplan Meier analysis of survival of 50 subjects with complete DiGeorge anomaly treated with thymus transplantation. Both typical and atypical subjects are included. Thirty six subjects survive.

Figure 7.

T cell outcomes after thymus transplantation. A) CD3, B) CD4, C) CD8, D), naïve CD4, and E) naïve CD8 T cells in subjects with typical complete DiGeorge anomaly treated with thymus transplantation without immunosuppression. The 10th, mean, and 90th percentiles are from Shearer et al.[22]

Figure 8.

Flow cytometry showing naïve CD4 T cells. CD4 T cells were analyzed with CD45RA and CD62L antibodies. The CD4 T cells in the upper right quadrant are naïve T cells. A) Healthy adult volunteer, B) Patient with atypical complete DiGeorge anomaly on day 271 after thymus transplantation.