CHAPTER 30 Thymus Transplantation

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History

Thymus transplantation was first attempted in the 1960s and 1970s using fetal thymus tissue [1, 2]. The results overall were disappointing [3-6]. In part the poor outcomes related to the lack of reagents needed to characterize and identify the patients into those who were truly athymic (complete DiGeorge anomaly) and those who had bone marrow stem cell problems (severe combined immunodeficiency). It is also possible that the fetal thymus tissue was too small to reconstitute a human infant [7]. The use of fetal thymus carried the risk of fatal graft versus host disease since mature T-cells can be found in the human thymus by the end of the first trimester [3]. By 1986, in a review of 26 infants treated with fetal thymus transplantation, 22 had died; the other 4 patients had achieved a 3-year survival [6].

Important research was conducted in animals in the 1970s and 1980s which would provide the background for improved outcomes. Hong and colleagues showed that thymus transplantation from completely mismatched mouse strains could reconstitute T-cells in nude mice [8]. In the 1980s and 1990s Haynes and colleagues performed animal experiments in which postnatal human tissue was transplanted into mice [9]. Dr. Haynes had reported in the early 1990s [9] that fragments of postnatal human thymus (readily available as discarded tissue from exposure of the heart in congenital cardiac surgery) could be transplanted in the SCID/human mouse model. If the mice were pretreated with an antibody against murine NK cells and macrophages, the human thymus tissue remained viable and murine T-cells colonized the thymus within 1-3 months. Human thymopoiesis did not develop as there was not a source of human stem cells.

In addition to the animal experiments that were essential for the development of the current thymus transplantation trials, other advances in the 1980s and 1990s allowed for critical advancement of the field. Dr. Haynes and other investigators developed monoclonal antibody reagents that identified components of the human thymus [10-12] and the earliest stages of human thymocyte development [13, 14]. In the 1990s, the ability to stain the cultured tissue for cytokeratin and other thymic elements allowed Markert and Haynes to develop culture conditions that maximized the viability of the cultured thymus slices [15]. Other monoclonal antibodies were developed to identify naïve T-cells [16]. This progress allowed accurate determination of the presence of thymically derived T-cells. At the same time the underlying immunodeficiencies were better defined. In particular, the difference between partial and complete DiGeorge anomaly was clarified, with the partial DiGeorge anomaly patients having a small thymus versus the complete DiGeorge anomaly patients having no thymus at all [17-19]. The former had some thymic-derived T-cells that could reject transplants. The latter did not have thymically derived T-cells; thus engraftment was facilitated.

Patient Population

The target population for thymus transplantation is the group of athymic infants with complete DiGeorge anomaly. DiGeorge anomaly is characterized by defects in organs derived from the 3rd and 4th pharyngeal pouches and the intervening 4th pharyngeal arch [20]. The parathyroid, thymus and heart are variably affected [19, 21-25]. Most infants have some parathyroid deficiency and require calcium replacement [26]. Typical heart defects include interrupted aortic arch type B and truncus arteriosus, although some patients have no cardiac defect at all [23, 26]. In complete DiGeorge anomaly, the thymus is absent. Other common problems in infants with complete DiGeorge anomaly include speech delay, aspiration, gastroesophageal reflux, rib or vertebral anomalies, renal abnormalities, atypical facies, developmental delay, hearing or visual deficits, 7th nerve palsies,

and cleft palate. Approximately half of children with complete DiGeorge anomaly have 22q11 hemizygosity [26-28]; approximately 20% have CHARGE association (*c*oloboma, *h*eart defect, choanal *a*tresia, growth or developmental *r*etardation, *g*enital hypoplasia, and *e*ar anomaly or deafness) [26, 29, 30] often with CHD7 mutations [31]; approximately 15% are infants of diabetic mothers [26, 32, 33]; and the remaining infants have no genetic or syndromic associations [26]. All athymic infants have a fatal condition and succumb to infection within the first 2 years of life because of their profound immunodeficiency [17].

Complete DiGeorge anomaly may present with two different phenotypes. The majority of infants have "typical" complete DiGeorge anomaly. These infants usually have very few T-cells (<50/mm^3) and always have fewer than 50 naïve T-cells/mm^3. Naïve T-cells are recent thymic emigrants that co-express CD45RA and CD62L [16]. Almost all of these infants will lack a proliferative response to the mitogen phytohemagglutinin (PHA) [17]. These infants do not have a rash. At some point after birth many infants with complete DiGeorge anomaly will develop circulating oligoclonal T-cells associated with rash and lymphadenopathy [34-36]. This phenotype is called "atypical" complete DiGeorge anomaly [34]. Patients with atypical complete DiGeorge anomaly resemble those with Omenn's syndrome [37-39]. The skin on biopsy shows spongiotic dermatitis with T-cell infiltration [34]. The T-cells appear to have developed without having been "educated". The oligoclonal T-cells seem to attack the infant and do not protect against opportunistic infections. These T-cells have infiltrated the liver, associated with hepatomegaly and elevated liver transaminases (unpublished). Strikingly, the oligoclonal T-cells can expand to very high numbers such as 40,000/mm^3 (unpublished). Despite the high T-cell numbers, less than 5% are naïve in phenotype. The peripheral T-cells may or may not proliferate in response to PHA. The two phenotypes of complete DiGeorge anomaly must be distinguished because the atypical patients can reject thymus transplants. Atypical patients require peritransplantation immunosuppression.

Screening of Recipients for Transplantation

Currently in the USA, an Investigator New Drug (IND) application with the Food and Drug Administration (FDA) is required for thymus transplantation. Because thymus transplantation is an experimental procedure, all transplantation is conducted under Institutional Review Board (Ethics Committee)-approved and FDA-reviewed protocols. Informed consent is obtained from parents of the donors and recipients prior to thymus transplantation.

The recipients are screened prior to transplantation to confirm the diagnosis of athymia and to better characterize the subject. For the diagnosis of athymia, the subject must have fewer than 50/mm^3 naïve T-cells in the peripheral blood on flow cytometry. In atypical complete DiGeorge patients with oligoclonal T-cells, less than 5% of circulating T-cells can be naïve in phenotype. Stimulation of peripheral blood mononuclear cells with the mitogen phytohemagglutinin is done to characterize the patient's T-cell response and determine if immunosuppression will be required. Every subject is tested for 22q11 hemizygosity.

In infants with rash and circulating T-cells, additional studies are performed. The clonality of the T-cells is assessed by flow cytometry and spectratyping [40]. T-cell receptor rearrangement excision circles (TREC) are quantified [41]. TRECs are episomes of DNA that form when the V, D, and J segments of DNA come together to encode the variable portion of the T-cell receptor chains. Absence of TRECs confirms the diagnosis of athymia made by the flow cytometry showing a lack of naïve T-cells.

In the atypical patients, maternal engraftment [42] and graft versus host disease (GVHD) from unirradiated blood transfusions must be ruled out. DNA is obtained from the infant's buccal swab and from the mother. The DNA samples are compared using molecular methods to DNA extracted from T-cells isolated from the infant's peripheral blood. GVHD from a blood transfusion is a life-threatening complication. The only infant who presented with GVHD in our series died despite intensive therapy to try to suppress the third party T-cells. Maternal cells are rarely seen in atypical complete DiGeorge anomaly. Their affect on transplant outcomes is not known at this time.

To prepare for transplantation, standard testing is conducted to assess the medical condition of the infant. Testing includes electrolytes, liver transaminases, renal function (creatinine, blood urea nitrogen, urinalysis, renal ultrasound), and HLA typing. A cardiac evaluation is performed to assess suitability for surgery. As for other transplant recipients, the subjects are screened for HIV-1 and hepatitis B and C. Subjects are also screened for human herpes virus 6 (HHV6), Epstein Barr virus (EBV), and cytomegalovirus (CMV). HHV6 may have a detrimental

affect on thymus development [43]. EBV and CMV are worrisome infections for infants with profound immunodeficiency as they can cause severe disease and may also be associated with lymphoproliferative disease [44-47]. Parents are counseled during the informed consent process that these infections may affect outcomes. If EBV or CMV is present, anti-viral therapy is instituted.

Infants are screened for autoimmune disease with complete blood counts, thyroid studies, Coombs antibody test, a urinalysis, and anti-HLA antibodies. If the subject has anti-HLA antibodies, the thymus used for transplantation cannot have the specific HLA antigens detected by the anti-HLA antibodies.

Donor Screening for Thymus Transplantation

The thymus tissue is obtained as tissue discarded during cardiac surgery. The surgeon removes thymus tissue to access the surgical field to improve the cardiac surgical outcome. The transplant team is called for all discarded thymuses. If an infant is awaiting thymus transplantation, the transplant team requests permission to approach the parents to obtain consent to use the discarded tissue for transplantation, to obtain blood and urine from the donor infant, and to obtain blood from the donor's mother.

The thymus donors are extensively screened for infectious diseases. The guidelines published by the FDA in the Code of Federal regulations are followed [48]. Thymus tissue is screened for hepatitis B, hepatitis C, EBV, and CMV by PCR. The thymus donor is screened for CMV infection by urine culture and by PCR of the blood. The donor is also screened for EBV by PCR of the blood. For donors over 1 month of age, additional screening is performed for HHV6 and West Nile Virus by PCR. All standard donor screening [48] is conducted on blood, including testing for hepatitis B and C, HIV-1, HIV-2, HTLV-1, HTLV-2, and syphilis.

The immune status of the donor infant is tested by flow cytometry to confirm normal percentages of total T, CD4, CD8, naïve CD4, naïve CD8, and B and NK-cells. Testing for 22q11 and HLA typing is performed on thymocytes that are harvested at the time of tissue slicing. Hemizygosity for 22q11 excludes a thymus donor.

The donor's parents are asked about autoimmune disease in themselves and the donor's siblings. Exclusion criteria include type I diabetes, thyroid disease, common variable immunodeficiency, lupus erythematosis, Crohn's disease, ulcerative colitis, and rheumatoid arthritis.

The donor's mother is tested for the same infections as the donor plus toxoplasmosis and Chagas disease. If the mother is IgG positive for toxoplasmosis, the infant is tested as well. The mother is tested for antibodies to CMV and EBV. Results consistent with acute infection lead to exclusion of the donor. In addition, extensive questionnaires are reviewed with the donor's parents to review risk factors for Creutzfeldt-Jacob disease, small pox exposure, severe acute respiratory syndrome (SARS) exposure, and West Nile disease exposure. Lastly, the mother is asked a series of lifestyle questions, including a sexual history to assess risk for HIV-1 and hepatitis.

Tissue Processing and Tissue Screening for Thymus Transplantation

The thymus tissue is brought to the laboratory and sliced aseptically using a Stadie-Riggs microtome [49] (Fig. 30.1). The slices are approximately 0.5-1 mm thick. Slices are placed on Millipore filters on surgical sponges which serve as rafts. Four filters are placed in each cell culture dish (Fig. 30.1) in thymus organ medium. The medium contains fetal calf serum, Hams F12, and HEPES [26]. The medium is aspirated daily and new medium is dripped onto the filters. This practice helps remove the T-cells from the tissue slices. All procedures are performed under Good Tissue Practices [50] under Standard Operating Procedures.

Donor tissue, thymocytes, and nucleic acid samples are stored on the day of harvest. These samples include DNA that can be used later to evaluate the recipient for graft versus host disease. Samples of tissue are stored frozen. Formalin fixed tissue is embedded in paraffin.

The identity of the tissue as thymus is confirmed by visual inspection and by immunohistochemistry from frozen or formalin fixed sections on the day of harvest, a culture midpoint approximately 3-7 days prior to transplantation, and on the day of transplantation.

Multiple sterility tests are performed. The culture medium is pooled on the day after harvest, at approximately 1 week prior to transplantation, approximately 3 days prior to transplantation and on the day of transplantation. The media are cultured for bacteria, fungus, and mycoplasma per the United States Pharmacopeia (USP). An endotoxin assay of pooled supernatant, using the limulus amoebocyte lystate as-

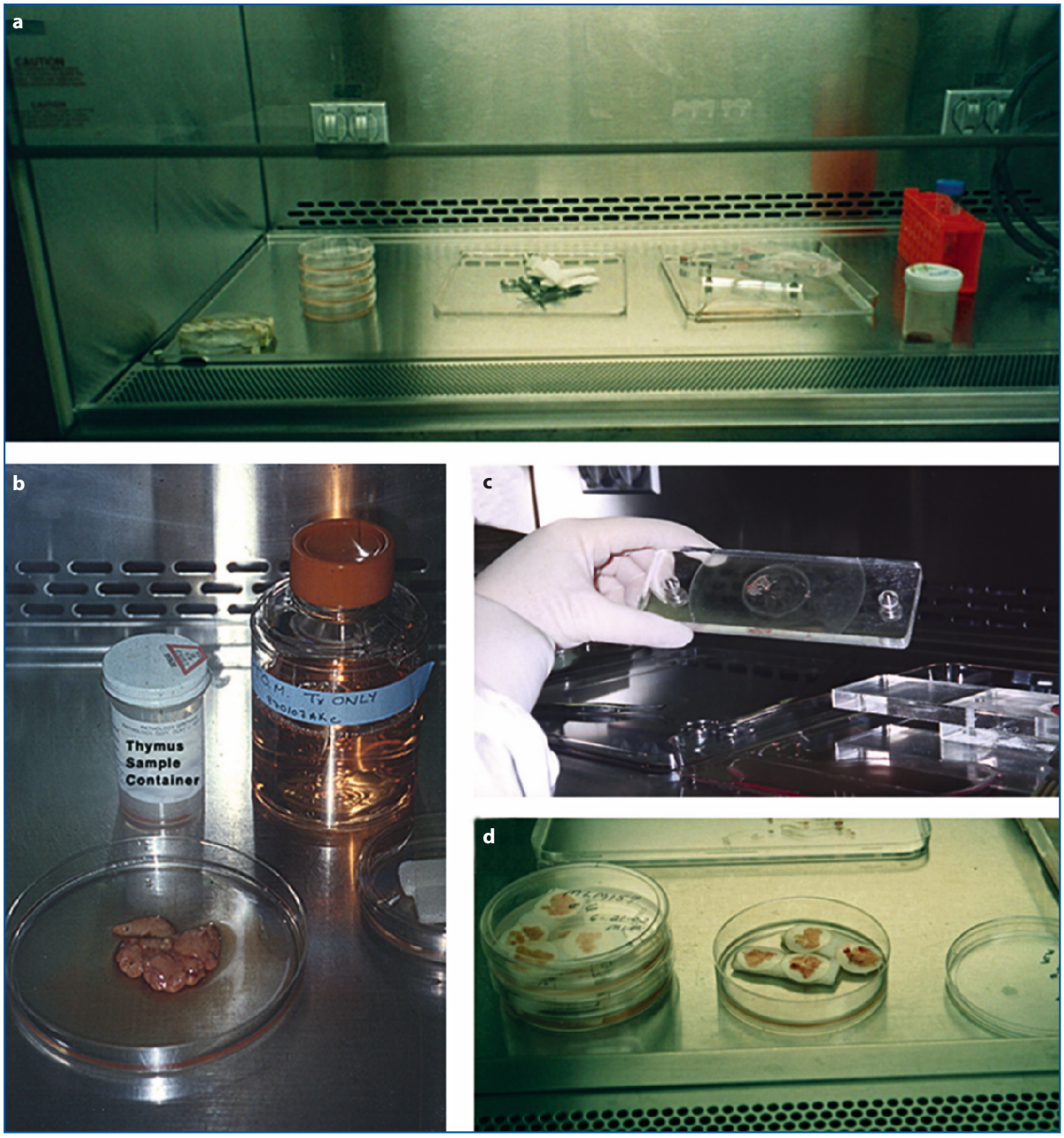

Fig. 30.1a-d Thymus processing. **a** Slicing equipment in the biosafety cabinet. Petri culture dishes are on the *left*, the thymus is on the *right* in the container. **b** The appearance of the thymus that is obtained from the operating room. **c** A slice of thymus. **d** Slices of thymus tissue that will be held in the tissue culture incubator until transplantation

say, is performed within 24 h of transplantation, and a Gram stain of pooled supernatant is assessed immediately prior to transplantation.

The dose of the thymus is ascertained the day prior to transplantation by physical measurement of all the pieces in each culture dish, estimating the length, width, and thickness of each piece. The dose range is between 4 and 18 g per meter squared of recipient body surface area.

Preparation of the Recipient for Transplantation

The initial immune screening of infants is used to determine if immunosuppression is required (reviewed in [26]). Infants with typical complete DiGeorge anomaly with low proliferative responses to PHA are not treated with immunosuppression before or after transplantation.

The use of PHA to determine the need for immunosuppression is problematic because PHA responses are not standardized. Three concentrations of PHA are used, and triplicate cultures of peripheral blood mononuclear cells are incubated for 3 days (or 3 and 4 days if sufficient cells are available). The highest proliferative result is used for the determination. Our laboratory uses a response of over 5,000 counts per minute (cpm) or greater than a 20-fold response over background as the threshold for requiring immunosuppression. Studies are underway to standardize the PHA assay so that responses are comparable.

All infants with atypical complete DiGeorge anomaly are treated with cyclosporine before and after transplantation. Steroids are added depending on the T-cell counts. In the 5 days prior to transplantation the atypical infants are treated with 3 doses of 2 mg/kg rabbit anti-thymocyte globulin concomitant with steroids, diphenhydramine, and acetaminophen.

Occasionally, subjects with typical complete DiGeorge anomaly have proliferative responses to PHA over 5,000 cpm and more than 20-fold over background. These subjects have very few naïve T-cells (<50/mm^3 or less than 5% of total T-cells). They do not have rash or lymphadenopathy. Because of the elevated proliferative response to PHA, they are also treated with rabbit anti-thymocyte globulin and the concomitant medications prior to transplantation. If the proliferative response reaches 75,000 cpm, they are treated with cyclosporine before and after transplantation as well.

Cyclosporine is carefully monitored to maintain trough levels between 180 and 220 ng/ml. Weaning of the cyclosporine (over 8-10 weeks) begins once naïve T-cells reach 5% of the total T-cells. A current focus of research is the management of atypical subjects who have detectable maternal T-cells in the circulation. Early weaning of the cyclosporine can lead to reappearance of the maternal T-cells in the blood. These maternal T-cells can threaten the integrity of the thymus allograft.

Surgical Procedure of Thymus Transplantation and Biopsy

Thymus transplantation is performed as an open procedure under general anesthesia in the operating room. As previously described [51], the thymus tissue is brought to the operating room in the dishes that have been used for culture. The tissue on the Millipore filters is transferred into sterile petri dishes on the operating table. The thymus tissue is placed into incisions in the quadriceps muscles bilaterally. Bleeding is minimized. Each piece is placed into its own pocket in the muscle and a suture is placed over the pocket to prevent the tissue from being extruding out of the muscle.

A biopsy of the transplanted tissue is performed 2-3 months after transplantation as an open procedure in the operating room. The initial incision is opened and the surface of the quadriceps muscle is accessed. Approximately four 5×5 mm pieces of tissue are obtained directly below 4 of the sutures that were used to close the muscle and that marked the location of the transplanted tissue. For each sample, part is frozen and part is placed in formalin and embedded in paraffin. Both parts are examined by immunohistochemistry. The presence of cytokeratin reveals the graft has been successfully sampled. Lymphocytes reactive with antibodies to CD3, CD1a, and Ki-67 (nuclear proliferation marker) are cortical thymocytes. The presence of these cells in the context of cytokeratin has been associated with development of naïve T-cells in the blood at 4-6 months after transplantation in all 23 biopsies with this finding to date. Figure 30.2 shows an allograft biopsy with cortical thymocytes and lacy thymic epithelium.

Clinical Outcomes

Subjects tolerate the surgical procedures with only occasional problems of dehiscence and inflammation around sutures. Figure 30.3 shows the Kaplan Meier survival estimate. Survival after transplantation is 73% with most deaths occurring in the first year after transplantation. These deaths are usually related to infection, cardiac, or pulmonary issues. One transplant recipient died of a sudden cardiopulmonary arrest 4 years after transplantation. The subject had undergone a second stage repair of her heart defect that had many complications 2 months prior to the event. It was assumed that the cause of death was a cardiac arrhythmia.

Prophylaxis for *Pneumocystis jarovecii* is stopped after the development of antigen-specific T-cell responses after 1 year. Discontinuing the prophylaxis has been possible in all subjects except for one who has ectodermal dysplasia and does not have T-cell proliferative responses to antigens. No subjects have developed pneumocystis infections after prophylaxis has been stopped.

Morbidity from infection is greatly reduced by 1 year after thymus transplantation. Viral infections that previously would have probably resulted in prolonged hospitalizations and that have resulted in the

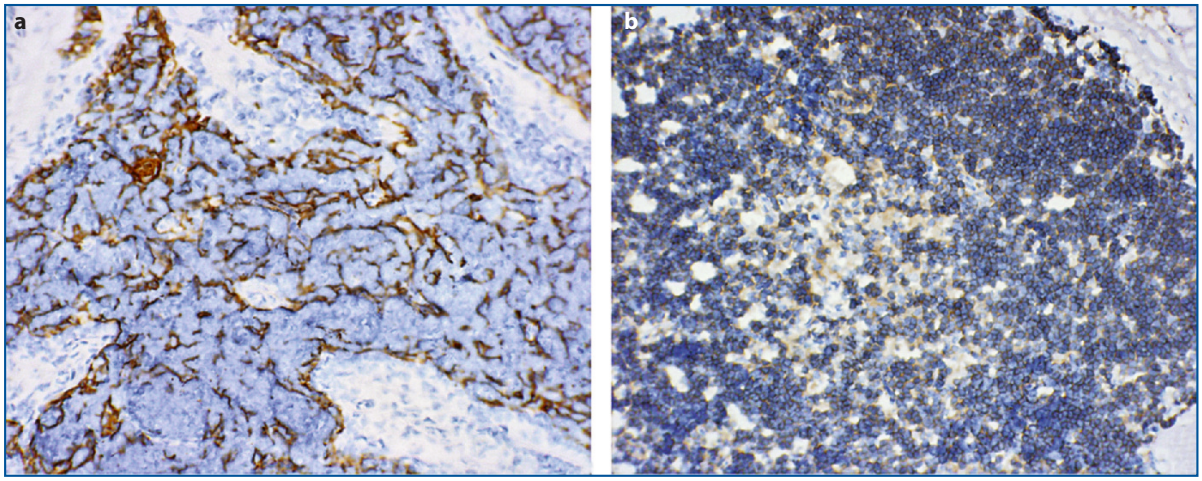

Fig. 30.2a,b Biopsy of thymus allograft. The biopsy from an atypical patient on day 66 after transplantation. **a** Cytokeratin. **b** CD3

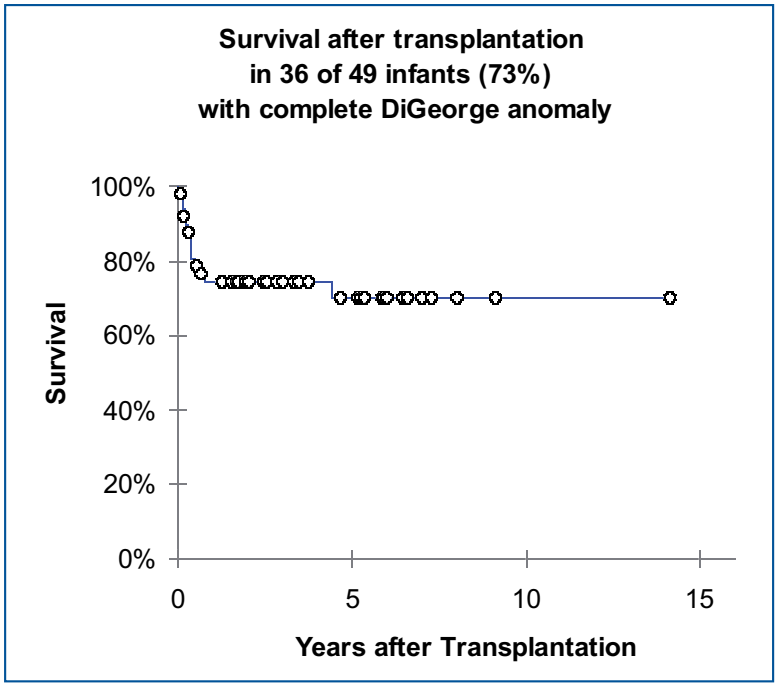

Fig. 30.3 Kaplan Meier Survival Function

death of patients (e.g., parainfluenza virus, RSV) have been associated with mild symptoms similar to those of other children, such as cough and fever. By 1 year, infections are usually followed in an outpatient setting without therapy. Problematic infections seen after 1 year are usually those caused by anatomic or neurologic problems, such as bronchomalacia leading to respiratory infections, abnormal ear and sinus anatomy (such as choanal atresia) resulting in otitis and sinusitis infections, and aspiration leading to recurrent aspiration pneumonias. The one exception in the author's series is one subject who had complete DiGeorge anomaly associated with ectodermal dysplasia. This subject developed naïve T-cells and a polyclonal repertoire but has not been able to mount antigen-specific responses. This subject has had significant viral infections requiring prolonged hospitalizations.

Autoimmune disease is a frequent complication after thymus transplantation found in 42% of subjects over 1 year post transplantation. Of the 31 infants who are more than 1 year after transplantation,

8 (26%) have developed autoimmune thyroid disease and are on replacement therapy. One of these infants developed alopecia totalis, which has not been responsive to therapy. A second of these infants with thyroid disease had previously developed nephrotic syndrome which responded to a 2-month course of steroids. (A renal biopsy was not performed.) Three additional subjects had transient episodes of thrombocytopenia. Two subjects developed autoimmune hemolytic anemia associated with viral infections. One of the subjects with anemia subsequently developed autoimmune hepatitis. These complications have responded to treatment. The one subject who developed autoimmune hemolytic anemia associated with HHV6 infection followed by autoimmune hepatitis continues on immunosuppression. These adverse events should be considered in the context of the high background of autoimmune disease in DiGeorge anomaly and in other primary immunodeficiencies such as severe combined immunodeficiency (SCID). Six subjects enrolled in transplantation protocols in the Duke series developed thyroid disease prior to transplantation. Thyroid disease has been described in partial DiGeorge anomaly [52-55] and in SCID after bone marrow transplantation [56]. Autoimmune cytopenias are a common and often severe complication of partial DiGeorge anomaly [57-59]. It is not clear at this time whether the autoimmune disease seen after transplantation is secondary to defective thymopoiesis or to the underlying genetic background of the recipient. Interestingly, neonatal thymectomy in an animal has been shown to increase the rate of spontaneous thyroiditis [60], suggesting that the mass of the thymus may be important for preventing thyroid disease.

The most severe adverse event occurred in the subject who received the largest dose of thymus tissue (23 g/m^2 body surface area). This subject presented with atypical complete DiGeorge anomaly. Most of the circulating T-cells were double negative ($CD3^+CD4^-CD8^-$). Large expansions of $TCRBV3^+$ T-cells were observed. Five months after transplantation, $CD4^+$ T-cells became the predominant population and the TCRBV repertoire normalized, suggesting that T-cells were emerging from the thymus. No naïve T-cells were detected. At the same time the subject developed severe unrelenting enteritis and colitis. This condition required high doses of immunosuppression to reverse. The subject died from a fungal infection, likely related to the steroid therapy. Because of this adverse event the maximum dose of tissue was lowered to 18 g/m^2 body surface area.

Immune Outcomes

All 31 subjects who are now more than 1 year after transplantation have developed naïve T-cells with the exception of DIG208 who remains on immunosuppression for autoimmune hepatitis (Fig. 30.4). Outcomes of CD3, CD4, and CD8 counts and PHA responses have recently been reported [26] and show improvements in all subjects. The CD4:CD8 ratio is normal in all subjects after transplantation. The CD4 and CD8 numbers are at approximately the 10th percentile for age. Naïve CD4 and CD8 numbers also increase to the 10th percentile for age (Fig. 30.4).

The T-cell receptor beta variable chain family repertoire normalizes after transplantation and has been reported for both typical patients [61] and atypical patients [40]. An example of normalization of repertoire in an atypical patient is shown in Fig. 30.5.

T-cell function, as assessed by proliferative responses to antigens and alloantigens, normalizes within the first 2 years of transplantation. Antigen-specific T-cell proliferative responses to tetanus toxoid have developed in all subjects after 1 year with the exception of DIG208 (who is still on immune suppression) and DIG017 (who has ectodermal dysplasia). Mixed lymphocyte reactions show normal responses to alloantigens. Of interest, the recipients are tolerant toward their thymus donor in mixed lymphocyte reactions [62].

B-cell function is tested 2 years after transplantation when replacement immunoglobulin is stopped. Table 30.1 shows serum immunoglobulin levels. For IgG, these values in the table are the most recent obtained after stopping immunoglobulin replacement. The IgA, IgM, and IgE values included in this table are the most recent levels obtained 1 year after transplantation. Serum IgG levels are low for age in only 1 of 18 subjects tested. Of note, the subject with the

Table 30.1 Serum Immunoglobulins in patients over 1 year after transplantation

Isotype	#	High	Normal	Low
IgG[1]	19	1/19 (5%)	18/19 (95%)	0/19 (0%)
IgA	31	4/31 (13%)	25/31 (81%)	2/31 (6%)
IgM	31	1/31 (3%)	25/31 (81%)	5/31 (16%)
IgE	30	6/30 (20%)	23/30 (77%)	1/30 (3%)

[1] Serum IgG values are for subjects more than 2 years after transplantation who are off immunoglobulin replacement therapy (n=22) for whom levels are available.

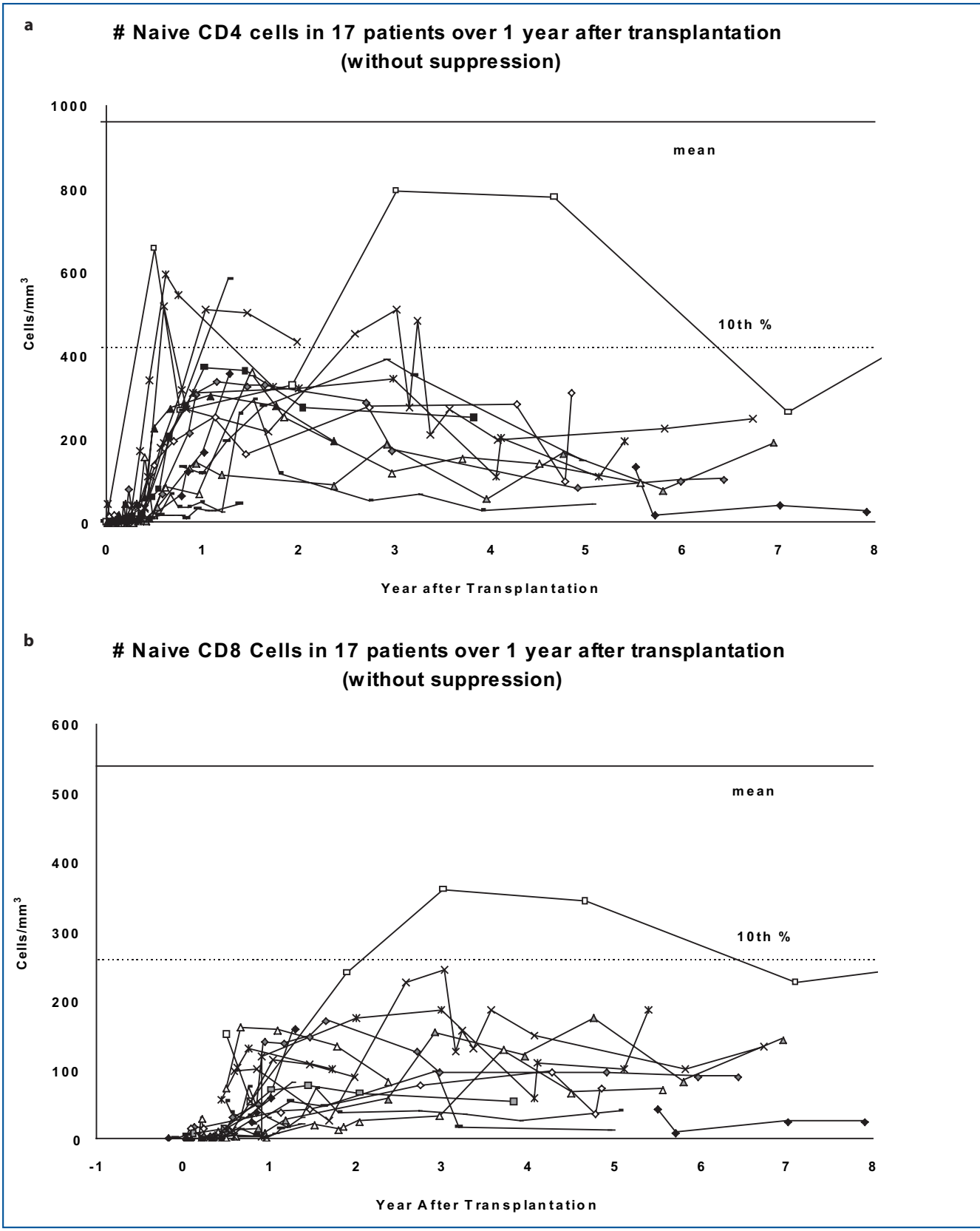

Fig. 30.4a-d Development of naïve T cells after thymus transplantation. The 10th percentile and mean for children aged 2-6 years are shown [65]. Each subject is an separate line

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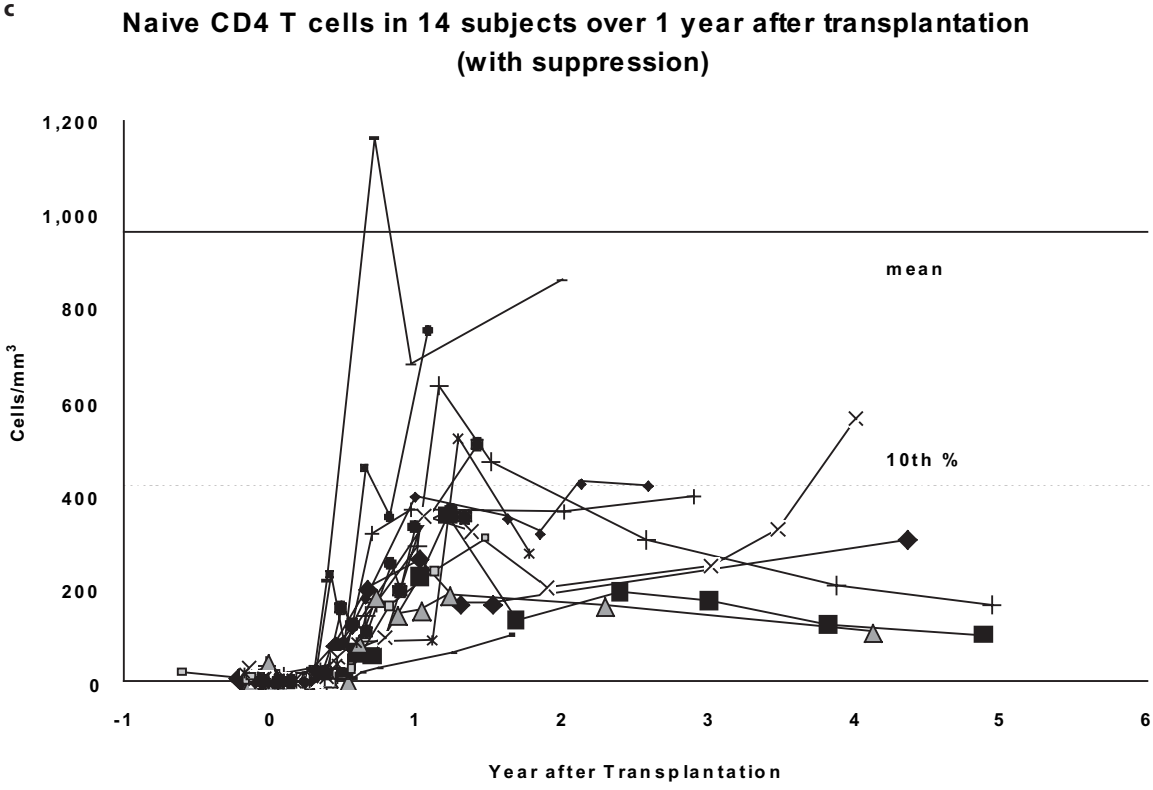
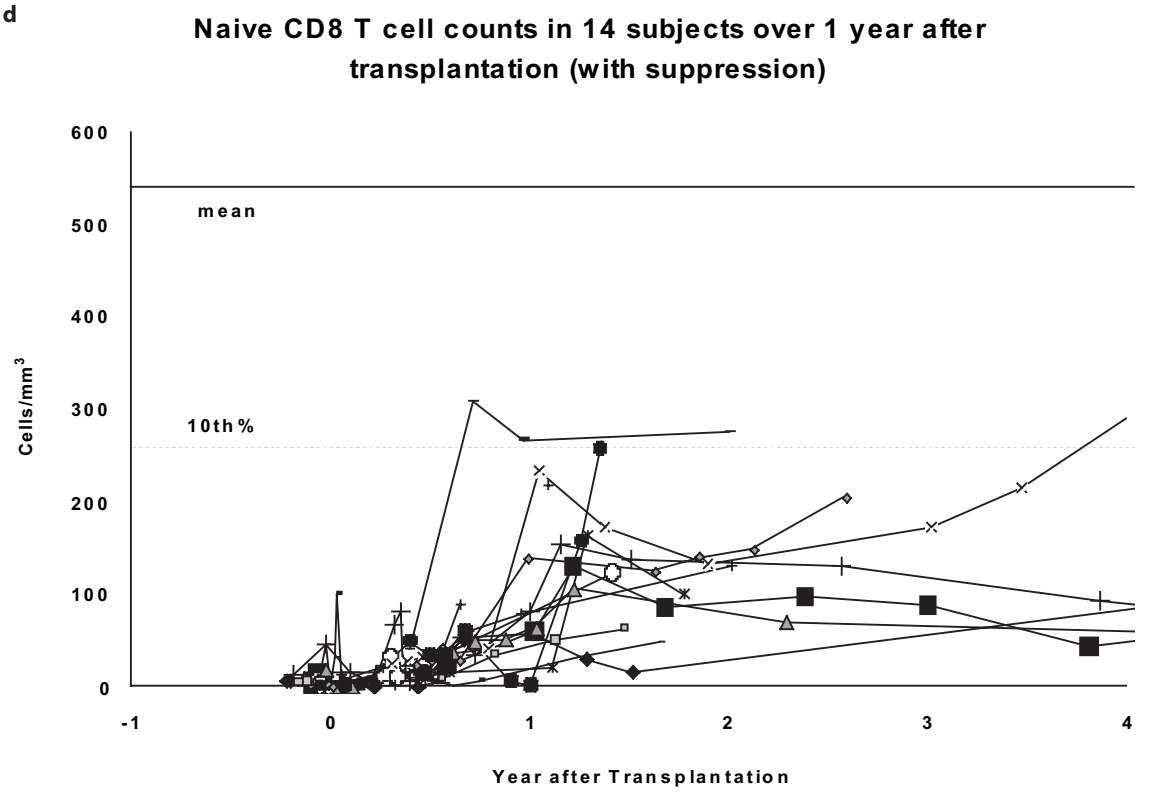

Naive CD4 T cells in 14 subjects over 1 year after transplantation (with suppression)
1,200
1,000
800
600
400
200
0
Cells/mm3
mean
10th %
-1
0
1
2
3
4
5
6
Year after Transplantation

d


Naive CD8 T cell counts in 14 subjects over 1 year after transplantation (with suppression)
600
500
400
300
200
100
0
Cells/mm3
mean
10th %
-1
0
1
2
3
4
Year after Transplantation

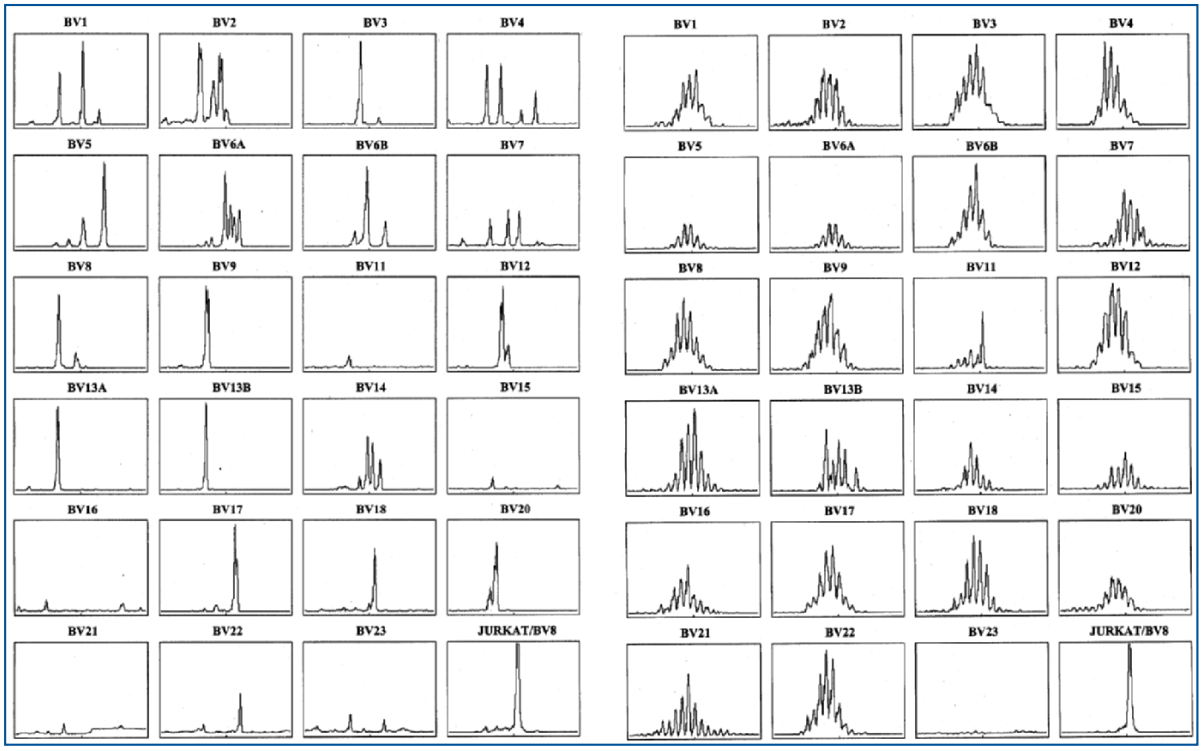


Fig. 30.5 Spectratype analysis of TCRBV diversity before and after transplantation in a subject with atypical complete DiGeorge anomaly. The panel on the *left* is day 19 with respect to transplantation. The panel on the *right* is day 301 after transplantation. Each profile represents a different TCRBV family. The *bottom right* profile in each panel is derived from Jurkat cells that express TCRBV8

Table 30.2 Specific antibody formation after thymus transplantation

Tetanus antibodies tested after 2 years (not done in 6)[1]	
Normal	17
Low	1
Pneumovax (CHO) antibodies after 2 years (not tested in 8)[1]	
Responses to 3 or more serotypes	14
Responses to 1-2 serotypes	2
No significant responses	0
Isohemagglutinins after 1 year (not done in 10)[2]	
Normal	7
Low	9
Negative	5

[1]24 subjects are over 2 years after transplantation. Twenty-two are off immunoglobulin therapy, 2 are on immunoglobulin therapy (one because of chronic aspiration). These antibody titers were obtained after immunoglobulin replacement was stopped.
[2]All values listed were obtained at least 1 year from transplantation in the 31 subjects over 1 year after transplantation

low IgG level makes antigen-specific antibodies [26]. IgA is elevated in 4 subjects. Two have CHARGE, one of whom has recurrent sinusitis secondary to choanal atresia. Another with elevated IgA has recurrent aspiration pneumonias. Only 2 subjects have low IgA despite the finding being common in partial DiGeorge anomaly [63]. IgE levels are elevated in 20%. Several subjects had low IgM levels, which was an unexpected finding although recently 3 subjects with 22q11 deletion were reported as having selective IgM deficiency [64]. Antibody titers are shown in Table 30.2. Tetanus toxoid and pneumococcal anti-carbohydrate antibodies to unconjugated vaccines are shown. These titers are obtained after immunoglobulin replacement is stopped. Most subjects generate normal antibody responses. The serum isohemagglutinins remain low or absent in a majority of subjects. This observation may correlate with the low IgM values in some subjects. Antibody formation is a current area of research.

Summary

Thymus transplantation is a promising therapy for the athymia of complete DiGeorge anomaly. The survival rate after transplantation is 73%, with follow-up as long as 14 years. Only one subject has died more than 1 year post transplantation. Excellent T- and B-cell function and a diverse T-cell repertoire develop in

most subjects. Over 90% are able to stop *Pneumocystis* prophylaxis and immunoglobulin replacement therapy. Immune reconstitution occurs when recipient T-cells develop in the donor thymus tissue. These recipient T-cells are tolerant toward the donor thymus. Autoimmune disease, especially thyroid disease and cytopenias, has been seen in a subgroup of subjects. The subjects continue to be followed for immune competence, thymic function, and adverse events.

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