Report of a Patient with T-Cell Deficiency and Normal B-Cell Function: A New Immunodeficiency Disease with Response to Transfer Factor

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An 11-yr-old boy had recurrent fevers and pulmonary infections since early childhood and, at age 7, had disseminated varicella with bilateral pneumonitis. A female sibling, age 1, died during this period of time with varicella pneumonia. Two years later, an immunological evaluation showed severe deficits in cellular immunity with skin anergy and very low or poor in vitro lymphocyte proliferative responses to mitogens, allogeneic cells and specific antigens. Quantitation of peripheral T-cells by spontaneous rosette formation was also low—40–45% (normal 61%). On the other hand, B-cell immunity seemed to be completely normal. Serum immunoglobulins and the immunoglobulin receptors on peripheral lymphocytes were normal. The patient produced specific antibodies upon antigen challenge (immunization) and after natural infection. Following transfer factor therapy, conversion of skin reactivity and clinical improvement occurred. No changes were seen in in vitro lymphocyte function with transfer factor therapy. Immunologic reconstitution persisted for 6 mo, after which the patient responded again to the administration of transfer factor. Although this patient has several characteristics in common with Nezelof's syndrome, the patient described in this report appears to represent a distinct clinical entity of primary isolated T-cell deficiency and normal B-cell immunity. The normal B-cell immune system, and the clinical and immunological response to transfer factor therapy, differentiates our patient from the syndrome of thymic dysplasia with immunoglobulin synthesis (Nezelof's syndrome).

INTRODUCTION

The greatest potential for transfer factor (TF) as a new mode of immunotherapy has been in the treatment of immune deficiency diseases. The advantages of TF therapy over other forms of immunological manipulation have been enumerated previously (1–3). In addition to TFs therapeutic potential, the mechanism of action of TF may be elucidated by studying patients with cellular immune deficiencies. Whether or not the patient responds to TF may also be useful in the classification of diseases which overlap in the spectrum of immune deficiency disorders.

We present herein a patient with an isolated primary T-cell deficiency who has responded to TF therapy. Although this patient shares many features with the

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diseases classified as Nezelof's syndrome of thymic dysplasia and immunoglobulin production (4), we believe the normal B-cell immune system and the response of the patient to TF separates our patient into a new entity of immunodeficiency.

**CASE HISTORY**

Patient D. W. was well until 6 mo of age, when multiple episodes of bronchitis, accompanied by patchy infiltrates on chest X-ray occurred. A sweat chloride test performed at age 2 was normal, as were subsequent sweat chloride determinations at ages 5 and 7. Between the ages of 2 and 4, he had recurrent fevers, cough, and wheezing often associated with patchy infiltrates on chest X-ray. An eczematoid rash was treated topically with steroid creams. A subsequent evaluation for allergy revealed intradermal sensitivities to grasses, molds, and house dust. Immunizations, including a smallpox vaccination at age 1, have been well tolerated.

At age 4, D. W. was admitted to the hospital with bilateral bronchopneumonia. Sinus radiograms showed bilateral maxillary sinusitis. Bronchiectasis of the left lower lobe was found at bronchoscopy.

At 7 yr of age, D. W. developed disseminated varicella with bilateral peribilar infiltrates and densities in both lower lobes. The skin lesions became secondarily infected with Staphylococcus, resulting in many keloidal scars and a bilateral cicatricial ectropion. Antibiotics and gamma globulins were used in therapy; recovery occurred over several months. A female sibling, age 12 mo, had varicella pneumonia and died during her acute illness. No other family member had similar problems or a history consistent with immunodeficiency.

During 1971, age 8, recurrent high fevers, a productive cough, and frequent episodes of bronchopneumonia characterized his clinical course following the severe systemic varicella infection. In August of 1972, D. W., age 9, was admitted to the University of Minnesota Hospitals for immunological evaluation. On admission, D. W. was in the 75th percentile for weight and height. Positive physical findings included extensive scarring and keloids of the skin secondary to the infected varicella lesions at age 7. Diffuse bronchi were audible in the chest, and mild hepatosplenomegaly was present. Small lymph nodes were palpable in the neck, axilla, and groin. Pulmonary function tests showed moderate obstructive lung disease with a slight arterial O_2_ desaturation (67% at room air). Radiographs showed a diffuse bilateral interstitial infiltrate in the lungs and a chronic maxillary sinusitis. Laboratory studies indicated normal liver and renal function, and normal blood chemistries. The hemoglobin was 14.1 g %; white cell count was 7200/mm^3 with eosinophilia (10–22%), and a platelet count of 140,000/mm^3.

Extensive immunological evaluation was followed by treatment with transfer factor.

**Histopathology**

Open liver and lung biopsies were performed. The liver architecture was normal, with a minimal mononuclear cell infiltrate in the portal areas. Mild interstitial fibrosis and a moderate mononuclear cell infiltrate into the alveolar septa were seen in the lung biopsy specimen. Large collections of mononuclear cells resembling lymphoid tissue were scattered throughout the lung parenchyma (Fig. 1). Occasionally, these areas contained plasma cells and primary lymphoid follicles. On
biopsy, a stimulated inguinal lymph node showed abundant germinal centers and plasma cells but lymphocyte depletion of the paracortical and deep cortical T-cell zones. Cultures of the tissues for virus, mycobacterium, and bacteria were negative.

MATERIALS AND METHODS

Skin test antigens included Candida (dermatophytin "O") $^1$ 1/10; mumps $^2$; purified protein derivative (PPD), $^3$ 2nd strength; and streptokinase-streptodornase (SK-SD), $^4$ 50 units. Skin reactivity was read at 24 and 48 hr as follows: 3 mm (−), 3–5 mm (±), 5–8 mm (1+), 8–12 mm (2+), 12–15 mm (3+). Dinitrochlorobenzene (DNCB) testing was performed as previously described (5). Each dose of TF was equivalent to $1 \times 10^9$ mononuclear cells and was prepared by methods previously described (6). The TF donors had positive skin reactivities to mumps, SK-SD, and candida.

Serum immunoglobulins (IG) were quantified by the single radial diffusion technique of Mancini. Immunoglobulin receptors on peripheral lymphocytes were carried out by methods described earlier (7), and quantitation of the antibody responses to diphtheria, tetanus, and pneumococcal antigens were done in the Clinical Immunology Laboratory (Dr. E. Yunis, Minneapolis).

$^1$ Hollister-Stiehr L., Spokane, Wash.
$^2$ Eli Lilly & Co., Indianapolis, Ind.
$^3$ Parke, Davis & Co., Detroit, Mich.
$^4$ Lederle Labs., Div. of American Cyanamid Co., Pearl River, N.Y.
Initial in vitro lymphocyte proliferative responses were performed by a macro-method described in detail elsewhere (8). Subsequent lymphocyte transformation tests were performed by a micromethod in which $2 \times 10^8$ lymphocytes (0.2 ml) separated by Ficoll–Hypaque gradient centrifugation were cultured in Micro test II plates (Falcon Plastics) with either mitogen or specific antigen (0.025 ml). Media RPMI 1640 supplemented with antibiotics, heparin, L-glutamine, Hepes buffer, and 15% pooled human serum was used to support cultures. Triplicate cultures with nonstimulated cultures as controls were incubated at 37°C in 5% CO$_2$ as follows: mitogens, 72 hr; mixed lymphocyte cultures (MLC), 5 days; and specific antigens, 6 days. Eighteen hours prior to harvesting, cultures were labeled with [methyl-$^3$H] thymidine, 0.5 $\mu$Ci (2.0 Ci/mM, New England Nuclear, Boston, Mass.). The incorporation of [$^3$H] thymidine was determined by liquid scintillation spectrophotometry.

Quantitation of T-cells in the peripheral blood by spontaneous rosette formation using nonsensitized sheep erythrocytes was performed as previously described (9). The normal mean for our laboratory is 61±3.2% (± 1 SD).

RESULTS

Immunological Studies

Serum immunoglobulins and the immunoglobulin receptors on peripheral lymphocytes were normal (Table 1). Isohemagglutinins were present, and the serum contained rheumatoid factor. The patient responded to stimulation with diphtheria, tetanus, and pneumococcal polysaccharide antigens with specific antibody formation. A 1:8 antibody titer to varicella–zoster undoubtedly reflects the varicella infection at age 7. The production of antibodies to rubella virus during a recent illness indicated an infection with this viral agent (Table 1). Serum complement studies were normal, as were neutrophil phagocytosis and opsonization.

The total lymphocyte count ranged from 1540 to 2700/mm$^3$. On admission the patient was anergic to all skin test antigens (Fig. 2) and showed negative skin reactivity to challenge with 100 pg of dinitrochlorobenzene (DNCB) on two occasions despite a successful sensitizing burn. T-cell quantitation of the peripheral lymphocytes by the rosette method with unsensitized sheep erythrocytes ranged from 40 to 45% (normal values at time of assay, 61±3.4%). In vitro lymphocyte proliferative responses showed particularly low responses to phytohemagglutinin (PHA), pokeweed mitogen (PWM) and allogeneic cells. Prior to TF therapy, poor responses were seen to several antigens in vitro. Lymphocyte mediator production was not investigated.

Clinical Course following Treatment with Transfer Factor

After four doses of TF, conversion of skin reactivity occurred to two of the three antigen markers present in the TF donor. Repeat challenge with DNCB (50 pg) resulted in a positive reaction. Although the TF donor denied previous contact with this chemical, he was sensitive to a challenge with 100 pg of DNCB when tested 6 mo after the preparation of TF. No changes were seen in the in vitro lymphocyte proliferative responses to mitogens or specific antigens.

One month after the completion of the first course of TF, the patient had a flu-like illness followed by idiopathic thrombocytopenia (ITP) which resolved without
TABLE 1
EVALUATION OF THE B-CELL IMMUNE SYSTEM

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>Immunoglobulin receptors on peripheral lymphocytes (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1440 mg%</td>
</tr>
<tr>
<td>IgM</td>
<td>117 mg%</td>
</tr>
<tr>
<td>IgA</td>
<td>92 mg%</td>
</tr>
<tr>
<td>IgE</td>
<td>20.7–IU</td>
</tr>
</tbody>
</table>

Autoantibodies
- Microsomal: neg
- Thyroid: neg
- Thyroglobulin: neg
- Rheumatoid factor: pos 1:320
- Antinuclear factor: neg
- Isohemagglutinins
  - Blood type O: anti A 1:8
  - Anti B: 1:16

Antigen stimulation/antibody titers to specific antigens
- Pneumococcal polysaccharide type III
  - Day 0: 1:4
  - Day 7: 1:16
  - Day 12: 1:64
  - 2 mo: 1:16
- Diphtheria: 1:320
- Tetanus: 1:10,240
- Varicella–zoster: 1:8
- Rubella
  - 12-20-72: 1:8
  - 3-1-74: 1:32
  - 7-9-74: 1:256

a Normal values: IgG 15.6% (14–19); IgM 6.5% (4.4–9.5); IgA 5.4% (3.6–9.4).

Treatment in 2 mo during this period. Lymphocyte cytotoxic antibodies, leuko-agglutinins, and Coomb’s test were all negative. Antiplatelet antibodies or antibody to TF could not be demonstrated by examining the release of platelet factor 3 from normal platelets after incubation with the patient’s serum alone or with the patient’s serum plus transfer factor (kindly performed by R. H. Astor, Milwaukee, Wis.).

Over the next 4 mo, D. W. did well clinically, with no episodes of fever or pneumonia, and he had a marked increase in exercise tolerance with less sputum production. Good pulmonary toilet particularly contributed to his definite pulmonary improvement. Booster injections of TF in May of 1973 augmented the delayed skin reactivity (Fig. 2). No changes were seen in the percentage of T-cell rosettes, or in the in vitro lymphocyte proliferative responses to the mitogens or specific antigens. No further episodes of thrombocytopenia were observed.

During the next 5 mo, D. W. continued to do well. Approximately 6 mo after his last injection of TF, D. W. had unexplained fevers, and subsequently, in the next few weeks developed increased sputum production with right lower lobe pneumonia. He was hospitalized, and despite antibiotic treatment, the pneumonia progressed to involve both lungs. He was transferred to Walter Reed Hospital in January 1974, where immunologic evaluation showed that the patient was again
anergic (Fig. 2). TF (two doses) was again administered with conversion of skin reactivity. *In vitro* lymphocyte responses to PHA, Con A and PWM were low (Fig. 3) and absent to specific antigen (Fig. 4) both before and after transfer factor therapy. T-cell rosetting remained unchanged. The pulmonary infiltrates resolved slowly over a 5-mo period. *Haemophilus influenza* (not group B) was the only bacterial organism cultured from sputum samples. The patient has remained clinically well, receiving weekly injections of small amounts of TF. At the last testing (Nov. 1974), skin reactivity has remained positive to all three antigens.

Fig. 2. Delayed skin reactivity and therapy with transfer factor (each dose equivalent to $1 \times 10^8$ mononuclear cells).
FIG. 3. *In vitro* lymphocyte responses to mitogens during the administration of transfer factor. 
a. Stimulation ratio: cpm stimulated cultures/cpm unstimulated cultures. b. PHA—phyto-
hemagglutinin-P, 10 μg/0.2 ml/2 × 10⁶ lymphocyte. c. Con—Concanavalin A—2 μg/0.2 
ml/2 × 10⁶ lymphocyte. d. PWM—pokeweed mitogen, 10 μg/0.2 ml/2 × 10⁶ lymphocyte.

FIG. 4. *In vitro* lymphocyte proliferative responses to specific antigens. a. Stimulation ratio: 
≥ 2.0 represents nonstimulation to specific antigen. b. SK/SD—streptokinase/streptodornase, 
5 units/0.2 ml/2 × 10⁶ lymphocyte. c. Candida—dermatophytin “O” (undiluted)—final concn. 
1/100/0.2 ml/2 × 10⁶ lymphocyte.
Family Studies

The father, mother, and two female siblings, ages 8 and 2, have been in good health. *In vitro* lymphocyte proliferative responses were normal to mitogens and allogeneic cells. Delayed skin reactivity and lymphocyte transformation tests were normal to SK/SD, mumps, and candida antigens in the father. No other testings were performed on the family.

DISCUSSION

In 1964 Nezelof et al. (4) described a family in which the propositus had severe deficiencies of cellular immunity and normal serum concentrations of immunoglobulin. These patients have been encountered by others (10-16) and have many characteristics in common with the syndrome of severe combined immunodeficiency (SCID). Nezelof's syndrome appears to be in most instances transmitted as an autosomal recessive genetic trait (17). Recurrent infections with viruses and gram negative bacteria, failure to thrive, and candidiasis have led to a fatal outcome, usually within the first 4 yr of life. These patients have lymphopenia and a dysplastic thymus which contains no Hassell's corpuscles. Plasma cells are found in the bone marrow and lymph nodes. Immunologically, T-cell function is severely deficient, with cutaneous anergy and absent or very poor *in vitro* lymphocyte responses to mitogens, allogeneic cells and specific antigens (17). Formation of spontaneous rosettes with unsensitized sheep cells is also very low (18). Although these patients seem to produce immunoglobulins, formation of specific antibodies upon antigen challenge is usually lacking (10, 19, 20).

Variability in the deficiency of the humoral and cellular immune systems occurs in Nezelof's syndrome, widening the spectrum considerably from that reported for Nezelof's original patient. Firman et al. (11), Fulginiti et al. (12), and Goldman et al. (19) have all reported patients with dysgammaglobulinemia. Heterogeneity is also found in the cell mediated immune responses. Delayed skin reactivity was found in the patient described by Goldman et al. (19). Ammann et al. (20), and Lawlor et al. (17) have reported several patients in whom the *in vitro* lymphocyte responses to PHA and/or allogeneic cells were not totally absent. Although patients with thymic dysplasia and immunoglobulin production (Nezelof's syndrome) have less severe immune deficits and survive longer than patients with SCID, considerable impairment of both the cellular and humoral immune systems can be demonstrated.

The genetics of the immune deficiency in our patient is not established, but the death of a female sibling at 1 yr of age with varicella pneumonia suggests an immunodeficiency with an autosomal recessive inheritance. Varicella-zoster infections occur in patients who are on immunosuppressive therapy (21, 22), or who have a compromised T-cell immune function (23). Although the histopathology of the thymus and immunological testing was not available for the female sibling in this family, a severe deficiency of T-cell immunity seems likely.

The histopathological findings in the lungs of our patient, showing lymphoid tissue invading the lung parenchyma, is most unusual. This type of lung pathology has been encountered to our knowledge in only one other patient with immunodeficiency disease. Dupree and Goldman (personal communication) had a child, whose clinical picture, immunological features of deficient cellular immunity with normal humoral immune function, and response to TF therapy was very similar to that
of our patient. Perhaps, the pathogenesis of this type of lung histopathology stems from excessive responses of the B-cell immune system to recurrent viral infections as compensation for the absence of a vigorous T-cell system. The idiopathic thrombocytopenia purpura, the positive serum rheumatoid factor, and the pathogenesis of the lung histopathology in our patient may indicate a hyperresponsive B-cell immune system in the absence of adequate T-cell immune control systems. Recently, an important control mechanism for B-cell function has been demonstrated for a population of T-cells (24). These T-cells, suppressor cells, exert feedback control on the synthesis of antibodies by B-cells. The failure of this control system of suppressor T-cell activity may be an important factor in the development of autoimmunity (25). Loss of T-cell suppressor function in New Zealand mice leads to autoimmune disease, i.e., Coombs' positive hemolytic anemia and immune complex glomerulonephritis (26). The T-cell suppressor function can be reconstituted with thymus cells from young mice (27). In certain primary, i.e., Wiskott–Aldrich syndrome (6), or acquired, i.e., systemic lupus erythematosus, immunodeficiency diseases, deficits of T-cell immunity responsible for T-cell control of B-cell expression may predispose these patients to the development of autoimmune diseases. Perhaps, reconstitution of their T-cell suppressor function with TF, thymus grafts, or thymosin could reestablish T-cell control and prevent autoimmune disease.

Our patient D. W. appears to represent a distinct clinical entity of primary isolated T-cell deficiency and normal B-cell immunity. Two major characteristics differentiate our patient from the patients with thymic dysplasia and immunoglobulin production (Nezelof syndrome): (1) normal functioning B-cell system and (2) clinical and immunological responses to TF therapy. Lymphopenia was never present in our patient. Plasma cells were abundant, and good germinal centers were present in the lymph nodes. A specific humoral response was demonstrated to several antigens upon challenge (immunization) and also following natural infection. The deficiency in cell mediated immunity in our patient was not as severe as that of most patients with thymic dysplasia. Lymphocyte responses in vitro to mitogens and to allogeneic cells in MLC were low, but they were present. Although the in vitro lymphocyte transformation studies did not change after TF therapy, this lymphocyte response might be dose-related. We have recently described such changes in lymphocyte transformation with higher doses of TF in patients with the Wiskott–Aldrich syndrome (6). Perhaps, the best differential point between our patient D. W. and the reported cases of thymic dysplasia syndrome was the clinical response and skin test conversion over several cycles of TF therapy. Recently, Lawlor et al. (17), and Pachman et al. (28) have reported that patients with thymic dysplasia and immunoglobulin production did not respond to TF. On the other hand, three of six patients reported by Ammann et al. (20), responded to TF with conversion of skin reactivity. All of these patients had some in vitro lymphocyte responses either to PHA and/or allogeneic cells prior to TF therapy, but one patient had a prolonged clinical effect. None of these patients responded to antigen challenge with specific antibody production.

Although the mechanism of TF remains to be elucidated, the success of TF therapy, particularly in patients with partial T-cell deficits, e.g., Wiskott–Aldrich syndrome (3, 29), chronic mucocutaneous candidiasis (3, 30–31), ataxia telangiectasia (32), and in our patient, suggests that a certain critical level of T-cell differentiation needs to be present. Interestingly, several patients with thymic dysplasia have responded to TF therapy transiently (17, 20). Often, skin test
conversion and, rarely, changes in in vitro lymphocyte responses occur, but clinical improvement has been very unimpressive, and the TF related lymphocyte changes were often short-lived. These patients have some residual functioning cellular immune system, as demonstrated by a very low PHA or MLC response, suggesting that a small subpopulation of functioning post thymic lymphocytes exists. However, because of a postulated stem cell defect in the patients with thymic dysplasia (33), and/or lack of thymic influence, i.e., thymosin (34), replenishment of this post-thymic subpopulation cannot occur. TF may then "deplete" this subpopulation by inducing maturation and terminal differentiation. If a large postthymic lymphocyte population is available, and means for replenishment of this population exist, a favorable clinical response to TF therapy can be expected. Furthermore, the response to TF therapy may be useful in the classification of the immune disorders which fall into the heterogeneous spectrum of T-cell deficiency. Thus, in our patient the response to TF therapy favors a defect in T-cell maturation beyond that of the defect in Nezelof's syndrome.

The mechanism(s) of TF activity in our patient may have been mediated through a cell population(s) other than the lymphocyte. Changes in T-rosetting or in vitro lymphocyte proliferative responses did not occur with TF therapy when conversion of delayed skin reactivity and clinical improvement were seen. Several observations have suggested that dialyzable TF may act on monocytes or macrophages in certain immunodeficiency disorders. Valdimarsson and co-workers (35) proposed that the response to TF in a patient with chronic mucocutaneous candidiasis (CMCC) of the granulomatous variety was related to the correction of a postulated macrophage defect. Following treatment with TF, a child with CMCC developed delayed hypersensitivity with restoration of a monocyte chemotactic defect (36). Nelson and Simmons (37) were able to obtain lymphocyte proliferative responses to candida antigen in vitro after the addition of TF in patients with CMCC. The impaired lymphocyte proliferative responses could also be corrected by the addition of macrophages to the cell cultures from normal individuals implying that TF corrected a macrophage "helper" defect (38). In addition, Valdimarsson (39) was able to demonstrate the enhancement of phagocytosis of yeast particles by monocytes after the addition of TF in vitro. These studies in patients with CMCC suggest that TF may act upon the macrophage and possibly have an important influence on macrophage–lymphocyte interaction. The study of patients with various types of immunodeficiencies offers an opportunity to elucidate the mechanism of action of TF and the cell population(s) upon which TF acts.

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