Successful restoration of immunity in the DiGeorge syndrome with fetal thymic epithelial transplant

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SUMMARY A 13-month-old girl presented with right upper lobe pneumonia and hypocalcaemic seizures: investigations showed hypoparathyroidism and impaired cell-mediated immune responses. Other features of the DiGeorge syndrome included hypertelorism, short philtrum of the lip, right-sided aortic arch, and aberrant origin of the left subclavian artery. Successful restoration of the immunodeficiency was achieved by transplantation of fetal thymic epithelium.

The DiGeorge syndrome is a congenital immunodeficiency disorder characterised by absence or hypoplasia of the thymus and parathyroid glands (DiGeorge, 1965, 1968). Soon after the description of the syndrome successful restoration of cell-mediated immunity was reported in 2 patients transplanted with fetal thymuses (August *et al.*, 1968; Cleveland *et al.*, 1968).

In order to reduce the risk of graft-versus-host disease in immune-deficient subjects (Ammann *et al.*, 1973; Pyke *et al.*, 1975), some workers have used thymuses enclosed in millipore diffusion chambers to prevent the egress of donor lymphocytes (Steele *et al.*, 1972; Frenkel *et al.*, 1974). Others have used lymphocyte-depleted thymic epithelium instead, and reported successful reconstitution of immunity in patients with severe combined immune deficiency (Hong *et al.*, 1976; Jones *et al.*, 1977). We describe a case of the DiGeorge syndrome successfully treated with thymic epithelium.

Case report

The patient was a product of a 37-week pregnancy and normal delivery; birthweight 2.4 kg. Except for a few 'colds', there were no serious infections during infancy. Immunisations with diphtheria-pertussis-tetanus and live polio vaccine were well tolerated. Her milestones were slightly delayed: she sat without support only at 10 months, and stood with support at 13 months. She was described as a 'jittery' and 'difficult' baby. The parents were healthy and non-consanguineous; the father was aged 25 years and

the mother 23 years. The only sibling died 6 days after birth from respiratory distress: birthweight 1 kg and gestation 28 weeks.

Our patient presented first at the age of 13 months with a right upper lobe pneumonia, with mild fever and respiratory distress. She weighed 8.3 kg, height was 74 cm, and head circumference 45 cm. The features were unremarkable, except for a short philtrum of the lip and mild hypertelorism (Fig. 1).

Hb was 13.0 g/dl, red cell count $5.01 \times 10^{12}/l$, WBC count $6.2 \times 10^9/l$ with 46% neutrophils, 6% eosinophils, 1% basophils, 29% lymphocytes, 17%

Fig. 1 Facial appearance of patient with the DiGeorge syndrome, showing hypertelorism and short philtrum.

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monocytes, and 1% plasma cells. Barium swallow showed a persistent filling defect at T3-T4 level and a right-sided aortic arch; this was confirmed by bronchoscopy. She was treated with antibiotics and physiotherapy and discharged after 2 weeks, clinically and radiologically well.

She was readmitted 2 days later with a generalised seizure lasting 3–4 minutes; another convulsion occurred the following day. Plasma Ca 1.20 mmol/l (4.8 mg/100 ml) (normal 2.25–2.65 mmol/l; 9–10.6 mg/100 ml), Mg 0.60 mmol/l (1.46 mg/100 ml) (normal 0.70–1.05 mmol/l; 1.7–2.5 mg/100 ml). Plasma concentrations of sodium, potassium chloride, glucose, urea, and serum albumin, globulin, and thyroxine were normal. A repeat estimation showed plasma Ca 1.30 mmol/l (5.2 mg/100 ml), Mg 0.60 mmol/l (1.46 mg/100 ml), and P 2.70 mmol/l (8.4 mg/100 ml) (normal 1.35–1.95 mmol/l; 4.18–6.04 mg/100 ml). These values were consistent with a diagnosis of hypoparathyroidism.

Correction of the hypocalcaemia was achieved by the use of AT 10 (vitamin D2) 1 ml daily, reducing to 0.5 ml daily, and calcium in the form of Sandocal 500 mg twice daily reducing to 500 mg daily. Plasma Ca and P gradually returned to normal. Liver function tests, cerebrospinal fluid, and microscopy of urine were normal. Electrocardiogram normal. Initial electroencephalogram (EEG) one week after her convulsions was reported as markedly abnormal: 'rhythmic high voltage 2–3 cycles per second delta waves predominated bilaterally posteriorly, while rhythms in the 5–6 cycle per second range were prominent in the central region'. These findings may merely have been postictal. A repeat EEG 2 months after her thymus transplant was considered normal. Developmental assessment suggested a delay of about 3 months behind chronological age.

Materials and methods

Immunological investigations were carried out by standard techniques: serum concentrations of immunoglobulin (A,G,M) by commercially available radial immunoassay plates (Behringwerke, West Germany); IgE by radioimmunoassay (Pharmacia, Sweden); lymphocyte transformation to mitogens and allogeneic cells by a microculture method (Thong *et al.*, 1973); T cells by E-rosette formation (Scheinberg *et al.*, 1976); B cells by surface immunoglobulins detected with polyvalent fluorescein-labelled antiserum (Cooper *et al.*, 1971). Biopsy of the left inguinal lymph node was performed 5 days after subcutaneous injection of 0.5 ml TAB vaccine.

For transplantation, a thymus was obtained from a 20-week-old fetus delivered by hysterotomy as a therapeutic procedure. It was cut into 1 cm size

pieces and pressed gently against a 250 μ m-size stainless steel mesh, washed thoroughly, and put into plastic tissue culture flasks containing RPMI 1640 medium with 10% fetal calf serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin. After 8 days at 37°C in a 5% CO₂-air atmosphere, the epithelium was removed and washed several times to get rid of any contaminating lymphocytes. It was minced into tiny pieces with sterile scissors. A half-thymus equivalent of this lymphocyte-depleted epithelium was divided into two portions; one was injected into the peritoneal cavity and the other into the right gluteal region with a 16-gauge needle.

Results

Serum immunoglobulin levels on first admission: IgG 9.4 g/l, IgA 0.5 g/l, IgM 2.25 g/l, and IgE 10.8 U/ml. T cells were 28% (normal 50–65%), B cells 40% (normal 15–25%), and ³H-thymidine uptake after stimulation of lymphocytes by phytohaemagglutinin (PHA) was 1742 cpm as compared with 611 cpm in unstimulated cultures. Skin testing showed negative delayed hypersensitivity response to candida antigen.

Histology of the inguinal node showed normal architecture but marked hypocellularity. Several small poorly formed follicles were present in the cortex, with inactive germinal centres containing no identifiable mitotic figures or macrophages and only a few large lymphoid cells. The postcapillary venules of the paracortex were prominent, but the adjacent tissue contained few small lymphocytes, large lymphoid cells, or mitotic figures. Plasma cells, eosinophils, and neutrophils were present, and histiocytes and reticulin cells prominent. No small lymphocytes were seen in the lumen of a post-capillary venule or passing through the wall of such a vessel. There was no clear demarcation between the hypocellular paracortex and medulla, and similar cells were seen in both areas. The sinuses contained predominantly histiocytes with some eosinophils and neutrophils. The presence of these cells is evidence of the recent antigenic stimulation. The presence of follicles in the cortex and numerous plasma cells throughout the node is indicative of normal B cell function. There were occasional large lymphoid cells and mitotic figures in the paracortex, suggesting some T cell functional capacity, but the appearance of the paracortex was indicative of a severe T cell defect (Fig. 2).

After transplantation of thymic epithelium, her lymphocytes showed marked response to PHA stimulation when tested one week later (Fig. 3). There was an increase, though transient, in E-rosette percentage in the second week, E-rosettes remaining

low throughout the period of follow-up. PHA responsiveness was maintained throughout and the response was excellent to mixed lymphocyte culture of 3471 cpm ^3H -thymidine uptake to heterologous cells compared to 400 cpm to autologous cells.

Thoracotomy performed at age 18 months (3 months after thymus transplantation) showed that an aberrant left subclavian artery arising from a right-sided aortic arch was responsible for the filling defect of the oesophagus on radiography. Thymic tissue could not be found in the mediastinum.

Fig. 2 Appearance of lymph node before thymus transplantation—specifically stimulated left inguinal node. The tissue was embedded in epoxy resin and the section is only 0.5 μm thick. (A) Low power view showing cortex and deeper areas. (H & E $\times 35$.) (B) High power view showing a postcapillary venule (PCV) and the adjacent tissue in the paracortex. Plasma cells, polymorphonuclear cells, histiocytes, and a few small lymphocytes are identifiable. A mitotic figure is seen (arrow) near the PCV. Hypocellularity of tissue is obvious. (H & E $\times 290$.)

A lymph node taken from the superior mediastinum at operation showed normal architecture and cellularity. In the cortex were many follicles with large active germinal centres having macrophages, large lymphoid cells, and mitotic figures. The paracortex showed much more cellularity than in the initial biopsy, with small lymphocytes predominating. Large lymphoid cells and mitotic figures were seen in the vicinity of most postcapillary venules, plasma cells in the paracortex and medulla, but eosinophils and neutrophils were sparse. Few histiocytes were identifiable in the sinusoids and these structures were much less conspicuous than in the initial biopsy. These features indicate greatly increased T cell functional capacity, especially as this lymph node was selected at random from a site not subjected to specific antigenic stimulation (Fig. 4).

Follow-up at age 22 months showed that lymphocyte PHA responsiveness was maintained, but E-rosettes were persistently below normal levels. She continued to be clinically well and free from respiratory infection. Developmental assessment suggested that she had caught up, although her communication was essentially nonverbal. This had happened despite a family break-up. Serum calcium levels were stable on treatment with vitamin D2 and oral calcium supplements.

Discussion

The aetiology of the DiGeorge syndrome is unknown. Its occurrence is generally sporadic, although familial transmission has been established in one case (Steele *et al.*, 1972). It has been postulated that some pathological event at the 6–10th week of embryonic life can account for most of the features of this syndrome (DiGeorge, 1965, 1968; Hitzig, 1973), as the thymus, parathyroid, and aortic arch

Fig. 3 Lymphocyte responsiveness to phytohaemagglutinin and T and B cell subpopulations before and after transplantation with fetal thymic epithelium.

Fig. 4 Appearance of lymph node 3 months after thymus transplantation—mediastinal node excised at the time of thoracotomy (exploration of vascular anomaly). The tissue was embedded in paraffin and the section is 5 μ m thick. (A) Low power view of cortex and paracortex. (H & E $\times$ 30.) (B) High power view of an area comparable to that in Fig. 1B. Recirculating lymphocytes are seen passing through the wall of a post capillary venule (PCV). Small lymphocytes are numerous and a large lymphoid cell can be seen (arrow). The increased cellularity is obvious. (H & E $\times$ 290.)

structures are being formed from outgrowths of the third and fourth pharyngeal pouches. In the same period, facial structures undergo rapid development.

Our patient presented with all the major features of the DiGeorge syndrome, including functional hypoplasia of the thymus and parathyroid glands, right-sided aortic arch, aberrant origin of the left subclavian artery, hypertelorism, and short philtrum of the lip. The unusual aspect of this case was the late presentation at the age of 13 months. Most cases present in early infancy with tetany and infection (Hitzig, 1973). Although the history of jittery symptoms and delayed milestones was highly suggestive of hypocalcaemia, tetanic seizures were absent. Further, her lymphocytes had minimal reactivity to PHA and there was some cellular activity (presumably T cell) in the paracortex of the initial lymph node biopsy. These findings suggest that parathyroid and thymic function were not completely absent.

The thymus plays an important role in development of the immune system (Miller, 1961; Martinez *et al.*, 1962). There is evidence that this influence is mediated by means of humoral factors elaborated by thymic epithelium (Trainin and Linker-Israeli, 1967; Goldstein *et al.*, 1972). Some believe that close contact between thymic epithelium and lymphocytes are important (Pyke *et al.*, 1975; Pyke and Gelfand, 1974). The use of thymic epithelium for

transplantation offers several advantages: the tissue can be injected with a syringe; since donor lymphocytes have been removed, there is virtually no risk of graft-versus-host disease, and transplanted thymic epithelium has been observed to attract host lymphocytes and assume the morphology of the thymus (Hong *et al.*, 1976) allowing direct lymphocyte-epithelium contact.

Thymus transplantation is not always successful in this syndrome. Patients can succumb to hypocalcaemia and its complications, or to serious infections before the benefits of immunological reconstitution are realised (Steele *et al.*, 1972; Touraine *et al.*, 1974). Failure to reconstitute immune responsiveness was noted in one case after three attempts with thymus transplantation (Astaldi *et al.*, 1977).

The results of thymus transplantation in our patient were gratifying. Lymphocyte responsiveness to PHA rose to normal levels within a week. Comparison of lymph node morphology before and after transplantation also showed marked improvement of histological appearance. We observed a transitory increase in the percentage of E-rosettes. Others have noted a gradual increase to normal levels (Good, 1976) or no increase (Cleveland and Glade, 1976). Good (1976) observed that immune reconstitution may be sustained for only a few years in some cases, and retransplantation may be necessary. However, the first patient ever to receive a thymus transplant continues to be immunocompetent after 9 years (Cleveland and Glade, 1976).

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References

- Ammann, A. J., Wara, D. W., Salmon, S., and Perkins, H. (1973). Thymus transplantation: permanent reconstitution of cellular immunity in a patient with sex-linked combined immunodeficiency. *New England Journal of Medicine*, **289**, 5-9.
- Astaldi, A., Astaldi, G. C. B., Wijermans, P., Groenewoud, M., Schellekens, P. Th.A., and Eijssvoegel, V. P. (1977). Thymosin-induced serum factor increasing cyclic AMP. *Journal of Immunology*, **119**, 1106-1108.
- August, C. S., Rosen, F. S., Filler, R. M., Janeway, C. A., Markowski, B., and Kay, H. E. M. (1968). Implication of a foetal thymus restoring immunological competence in a patient with thymic aplasia (DiGeorge's syndrome). *Lancet*, **2**, 1210-1211.
- Cleveland, W. W., Fogel, B. J., Brown, W. T., and Kay, H. E. M. (1968). Foetal thymic transplant in a case of DiGeorge's syndrome. *Lancet*, **2**, 1211-1214.
- Cleveland, W. W., and Glade, P. R. (1976). The first thymic transplant: a progress report (abstract). *Pediatric Research*, **10**, 384.

- Cooper, M. D., Lawton, A. R., and Bockman, D. E. (1971). Agammaglobulinaemia with B lymphocytes: specific detection of plasma cell differentiation. *Lancet*, **2**, 791-795.
- DiGeorge, A. M. (1965). A new concept of the cellular basis of immunity (discussion). *Journal of Pediatrics*, **67**, 907-908.
- DiGeorge, A. M. (1968). Congenital absence of the thymus and its immunologic consequences: concurrence with congenital hypoparathyroidism. In *Birth Defects*, Original Article Series 4, No. 1, pp. 116-123. Williams and Wilkins: Baltimore.
- Frenkel, L. D., Thong, Y. H., Steele, R. W., and Bellanti, J. A. (1974). Familial immune deficiency and leukodystrophy: reconstitution with fetal thymus in a Millipore chamber (abstract). *Pediatric Research*, **8**, 413.
- Goldstein, A. L., Guha, A., Zatz, M. M., Hardy, M. A., and White, A. (1972). Purification and biological activity of thymosin, a hormone of the thymus gland. *Proceedings of the National Academy of Sciences of the USA*, **69**, 1800-1806.
- Good, R. A. (1976). Immunodeficiency in developmental perspective. In *Textbook of Immunopathology*, pp. 555-606. Edited by P. A. Miescher and H. J. Muller-Eberhard. Grune and Stratton: New York.
- Hitzig, W. H. (1973). Congenital thymic and lymphocyte deficiency disorders. In *Immunologic Disorders in Infants and Children*, pp. 215-235. Edited by E. R. Stiehm and V. A. Fulginiti. Saunders: Philadelphia.
- Hong, R., Santosham, M., Schulte-Wissermann, H., Horowitz, S., Hus, S. H., and Winkelstein, J. A. (1976). Reconstitution of B and T lymphocyte function in severe combined immunodeficiency disease after transplantation with thymic epithelium. *Lancet*, **2**, 1270-1272.
- Jones, J. F., Sieber, O. F., Pinnas, J., Hong, R., and Fulginiti, V. A. (1977). Immunoglobulin production in severe combined immunodeficiency disease (SCID) after thymus epithelial explant therapy (abstract). *Pediatric Research*, **11**, 488.
- Martinez, C., Kersey, J., Papermaster, B. W., and Good, R. A. (1962). Skin homograft survival in thymectomized mice. *Proceedings of the Society for Experimental Biology and Medicine*, **109**, 193-196.
- Miller, J. F. A. P. (1961). Immunological function of the thymus. *Lancet*, **2**, 748-749.
- Pyke, K. W., Dosch, H.-M., Ipp, M. M., and Gelfand, E. W. (1975). Demonstration of an intrathymic defect in a case of severe combined immunodeficiency. *New England Journal of Medicine*, **293**, 424-428.
- Pyke, K. W., and Gelfand, E. W. (1974). Morphological and functional maturation of human thymic epithelium in culture. *Nature*, **251**, 421-423.
- Scheinberg, M., Blacklow, N. R., Goldstein, A. L., Parrino, J. A., Rose, F. B., and Cathcart, E. S. (1976). Influenza: response of T-cell lymphopenia to thymosin. *New England Journal of Medicine*, **294**, 1208-1211.
- Steele, R. W., Limas, C., Thurman, G. B., Scheulein, M., Bauer, H., and Bellanti, J. A. (1972). Familial thymic aplasia; attempted reconstitution with fetal thymus in a Millipore diffusion chamber. *New England Journal of Medicine*, **287**, 787-791.
- Thong, Y. H., Steele, R. W., Vincent, M. M., Hensen, S. A., and Bellanti, J. A. (1973). Impaired *in-vitro* cell-mediated immunity to rubella virus during pregnancy. *New England Journal of Medicine*, **289**, 604-606.
- Touraine, J. L., Incefy, G. S., Touraine, F., L'Esperance, P., Siegal, F. P., and Good, R. A. (1974). T-lymphocyte differentiation *in vitro* in primary immunodeficiency disorders. *Clinical Immunology and Immunopathology*, **3**, 228-235.
- Trainin, N., and Linker-Israeli, M. (1967). Restoration of immunologic reactivity of thymectomized mice by calf thymus extracts. *Cancer Research*, **27**, 309-313.

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