Immunological Studies in Congenital Nephrosis*

The nephrotic syndrome of infancy is a well-recognized though rare clinical entity (Hallman and Hjelt, 1959). The various clinical and morphological manifestations of this syndrome have been the subject of several publications (Lange et al., 1963; Royer et al., 1963; Hoyer et al., 1967). The pathogenesis of this entity is unknown; some investigators have suggested that immune mechanisms play a role (Kouvalainen et al., 1962; Kouvalainen, 1963; Lange et al., 1963). The purpose of this communication is to report a case of congenital nephrotic syndrome and the results of immunological studies performed to determine whether an autoimmune, immune complex, or graft versus host (GVH) reaction could be implicated in the etiology of the disease in this case.

Case Report and Methods

A 6-week-old Caucasian male of northern European descent was referred to the University of California Center for the Health Sciences in January 1971 for evaluation of proteinuria and anasarca which developed at 2 weeks of age.

The infant was the product of a 19-year-old, gravida 3, para 2, female whose pregnancy was uncomplicated. Labour and delivery at 36 weeks gestation were uneventful. Birth weight was 2.15 kg.

At 2 weeks of age periorbital oedema developed and progressed to anasarca. At 5 weeks of age the patient was admitted to a community hospital in Hemet, California where anasarca, massive proteinuria, hypoalbuminaemia, and a heart murmur were noted. Electrocardiograms and radiological studies of the cardiopulmonary system were normal. Cardiac catheterization was consistent with mild peripheral pulmonary coarctation. Because of progressive anasarca and hypoalbuminaemia (0.9 g%) the patient was referred to the UCLA Medical Center for evaluation at 6 weeks of age.

Physical examination revealed a pale infant with generalized oedema. Temperature was 37°C, heart rate 160 per minute and respirations were 28 per minute. The infant weighed 3.2 kg. Funduscopic examination was normal. Examination of the lungs was normal. A grade II/IV systolic murmur was heard at upper left sternal border, radiating towards both axillae. The liver was palpated 2 cm below the right costal margin.

The mother was a healthy housewife who had 2 healthy children, aged 2 and 4 years by a previous marriage. The father was a 25-year-old healthy lumber mill worker who had no previous children. There was no family history of renal disease, syphilis, allergic diathesis, or exposure to known nephrotoxic agents. The mother had no history of blood transfusions or injections of gamma globulin. There was no history of consanguinity in the family.

Initial Laboratory Data. Haemoglobin was 8.7 g%, red blood cell packed cell volume was 23%. White blood count revealed 6000 cells/mm³, 23% of which were segmented neutrophils; 67% lymphocytes, 5% monocytes, 4% eosinophils, and 1% basophils. Urinalysis revealed no reducing substances or acetone, the urine pH was 5.0, qualitative protein (sulphosalicylic acid method) 4+. Microscopic examination of the urinary sediment revealed numerous red blood cells, 6–8 white blood cells per high power field and 10–12 granular casts per high power field. Serum sodium was 143 mEq/l, potassium 4.2 mEq/l, chloride 98 mEq/l, creatinine 0.9 mEq/l, carbon dioxide 27 mEq/l, calcium 8.0 mg%, bilirubin 0.5 mg%, total protein 3.4 mg%, albumin 1.7 mg%, cholesterol 166 mg%, alkaline phosphatase 20 K-A units, serum glutamic oxaloacetic transaminase 66, serum glutamic pyruvic transaminase 17. Creatinine clearance was 17.2 ml/min per 1.73 m². Quantitative urine protein was 2.2 g/24 hours. Glomerular permeability index (Schreiner, 1960) was 2.48. Urine cultures were negative. Urinary amino acids were normal.

Radiological examination of the skull and long bones were normal. Serologic tests for syphilis (Venereal Disease Research Laboratories, FTA, TPI) on the parents and infant were negative as were Sabin dye tests, toxoplasma haemagglutination, and rubella haemagglutination. FTA IgM was negative on the infant.

Immunoglobulins and βC globulin, measured by radial immunodiffusion, revealed an IgG level of less than 100 mg%, IgM 135 mg%, IgA 38 mg%, and βC globulin 86 mg%.

HLA typing of lymphocytes and crossmatching studies and microdroplet cytotoxicity test were performed by Dr Paul Terasaki using the methods of Terasaki and McClelland (1964) and Terasaki (1970). Transformation of the infant’s lymphocytes in the presence of autologous serum, the mother’s serum, and homologous ABO and Rh-compatible serum and fetal calf serum, as well as transformation of the mother’s lymphocytes and homologous lymphocytes in the presence of autologous, homologous and the infant’s and fetal calf serum were studied by the incorporation of H²-thymidine into DNA both in the presence and absence of phytohaemagglutinin* (PHA) using the method of Rode and Gordon (1970). Mixed lymphocyte cultures between mother and infant were also performed.

Following transfusion with packed red cells and diuresis with intravenous ethacrynic acid, an open surgical renal biopsy was performed. One portion of cortex was placed in formalin and 4 micron sections stained for light microscopy with haematoxylin, eosin, and periodic

Received 6 December 1971.

* Supported in part by a Grant in Aid from the American Heart Association and the Southern California Kidney Foundation.
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acid Schiff. The other section was placed in a vial of isopentane which was immersed and stored in liquid nitrogen before immunohistological studies. Immunofluorescent studies were performed by direct staining with fluorescein isothiocyanate (FITC) conjugated rabbit antisera to human IgG, IgM, C3, and fibrinogen. These methods have been described previously (McIntosh et al, 1971).

The patient was treated with cyclophosphamide (2 mg/kg/day for 4 months) without improvement of creatinine clearance or decrease in proteinuria. He is now on diuretics and is doing poorly.

Results

Renal Morphology. The glomeruli showed minimal patchy basement membrane thickening and minimal focal segmental hypercellularity. The interstitium was oedematous with focal patchy monocytic inflammatory infiltrate and fibrosis. A few tubules were dilated or atrophic.

Immunohistology. Neither IgM, IgG, C3, nor fibrinogen were localized on the patient's kidney.

HLA Typing. Histocompatibility as graded by Dr Terasaki was B+ indicating no major group mismatch. Circulating antibody to histocompatibility antigens were not detected in mother or infant. Crossmatch was negative.

Lymphocyte Transformation Studies. There were no significant differences in the PHA response of the infant's lymphocytes in either autologous serum, homologous serum, or the mother's serum. The same was true of the mother's peripheral lymphocytes and homologous lymphocytes.

Mixed lymphocyte culture between the mother's and the infant's cells did not show increased thymidine uptake over control cultures.

Discussion

The nephrotic syndrome presenting in early infancy has been well recognized. Clinical and morphological studies suggest that this entity differs from other forms of nephrotic syndrome.

Although a few infants have been reported to have congenital syphilis and respond to penicillin therapy (Pollner, 1966), the aetiology and pathogenesis of this disease is usually obscure.

Congenital nephrotic syndrome has an especially high incidence in Finland. In studies from this country an autosomal recessive inheritance pattern has been well documented.

Low birth weights, prematurity, and large placentas are common. Polycythaemia, delayed osseous maturation, and elevation of serum IgM levels may also occur. Proteinuria is unaffected by steroid therapy and the outcome is usually fatal. The nature of the proteinuria and the use of cyclophosphamide therapy has not been previously reported in these patients.

Morphological findings are variable and include microcystic disease of the kidney, glomerular proliferation, hyalinization, basement membrane thickening, interstitial inflammation, and scarring (Hallman and Hjelt, 1959; Hoyer et al, 1967).

Some investigators have suggested that immune mechanisms may play a role in pathogenesis of this syndrome. This hypothesis is supported by reports of localization of IgG and β1C on glomeruli (Lange et al, 1963). The finding of rapid rejection of skin grafts from baby to mother prompted investigators to suggest that maternal sensitization to fetal antigens may play a role in this syndrome (Kouvalainen et al, 1962; Kouvalainen, 1963). These immunological findings were not confirmed in a study of 4 cases by Hoyer et al (1967).

Our detailed investigations have not elucidated the mechanisms involved in the development of this disease. Studies failed to show an infectious aetiology. The immunohistological data and the complete immunological studies do not support an immune mechanism. Because of the autosomal recessive inheritance pattern in this disease, a biochemical defect seems more likely.

Summary

A case of nephrotic syndrome of infancy is reported. Clinical and morphologic studies confirm the opinion that this entity differs from other forms of nephrotic syndrome. A search for known infectious agents was unrewarding and treatment with cyclophosphamide was not beneficial. Renal immunofluorescence studies were negative. The mother's serum showed no cytotoxic effect on the infant's lymphocytes in vitro and two-way mixed lymphocyte culture between the infant's cells and the mother's cells showed no increase in thymidine uptake over controls. These findings support the hypothesis that immune mechanisms did not play a role in this patient's disease. Because of the autosomal recessive inheritance pattern in this disease a biochemical defect seems more likely.

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Multiple Congenital Defects Associated with an Abnormal Unclassifiable Karyotype

We report the case of an infant with multiple congenital anomalies, and a karyotype that we were unable to classify.

Case Report

The propositus was born to a 29-year-old, gravida 5, para 4, mother after 42 weeks of uncomplicated gestation by Caesarean section because of malposition and fetal distress. One-quarter of the placenta had been aborted. The infant’s Apgar score at 1 min and again at 5 min was 2. The birth weight was 4120 g, length 53 cm, head circumference 39-5 cm, 5-5 cm greater than the chest circumference. He appeared lethargic, limp, and had a feeble cry. He had a wide forehead, orbital hypertelorism, and a capillary haemangioma near the right eyebrow. The palpebral fissures pointed outwards and upwards (Fig. 1). Grey-white opacities mottled both corneas. He had coloboma of the left iris and choroid. The left pupil was dilated; neither pupil reacted to light. The optic fundi were normal. The ears were low-set and poorly developed, the base of the nose depressed, the chin receding. The heart was enlarged. A grade I/IV systolic murmur was heard at the left sternal border. The liver and spleen and both kidneys were palpable. External genitalia were normal male. The limbs were normal.

Laboratory Investigations. The peripheral blood count, blood urea nitrogen, blood sugar, serum calcium, phosphorus, sodium, potassium, and chloride were normal. An electrocardiogram was read as normal. Urinary amino-acid chromatogram was also normal.

Radiological Investigations. The vault of the skull was enlarged, the sutures widened indicating increased intracranial pressure. X-rays of the chest showed marked cardiomegaly and pulmonary vascular congestion. Bilateral gross hydronephrosis and hydro-ureter were shown on IVP. A cystogram showed wide dilatation of the bladder neck and prostatic urethra.