PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFICIENCY: A THERAPEUTIC CHALLENGE

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Purine nucleoside phosphorylase (PNP:EC 2.4.2.1) deficiency appears to result in a predominantly T cell immune defect. Lack of the PNP enzyme leads to the inability to degrade deoxynucleosides, particularly deoxyguanosine (dGR) resulting in the intracellular accumulation of deoxyguanosine triphosphate (dGTP) which is known to inhibit DNA synthesis in vitro. Deoxycytidine kinase is considered responsible for the initial conversion of dGR to dGMP. Hence deoxycytidine (dCR), the preferred substrate, should competitively inhibit dGTP accumulation as has been demonstrated in vitro. However, oral dCR therapy for six months in a PNP deficient child produced no clinical or immunological improvement, probably due to degradation in the gut or rapid deamination.

We recently demonstrated total PNP deficiency shortly after birth in the second child of a family in which the first U.K. homozygote to be identified had died of a lymphoproliferative disorder.

This paper presents clinical, immunological and biochemical studies over twenty months in which specific therapeutic approaches were designed to try to prevent the progressive attrition of T cell function.

CASE HISTORY

A male infant (S.B.) was born at term in June 1980 to healthy parents who are fourth cousins. The child was physically and neurologically normal at birth with total PNP deficiency being confirmed in red and white cell lysates at four days of age. He has thrived with full recovery from urinary tract, respiratory
syncytial virus and Bordetella Pertussis infections. Oral thrush infection occurred for the first time at 16 months of age.

Head lag and excessive irritability were noted at three months of age. Since then the infant has remained profoundly hypotonic and developmentally delayed with recent hypertonia in the lower limbs.

METHODS

**Immunological** investigations were carried out using standard techniques on lymphocytes separated by Ficoll-Triosil gradient. T-cell subpopulations were detected by OKT4,6 and 8 sera (ortho). PHA responses, immunoglobulin levels and other standard tests of lymphocyte and also leucocyte function were measured by established methods.

**Biochemical** methods used for purine levels and enzymes are referenced in detail in a previous publication. Due to the lymphopenia 'lymphocyte' (peripheral blood mononuclear cell: PBM) nucleotide levels were not obtained until 9 months. High pressure liquid chromatography (HPLC) was used for the estimation of intracellular nucleotide and nucleoside levels.

**Therapeutic regimes** were divided into four separate periods. The first comprised deoxycytidine initially intravenously and then subcutaneously in a dose of 15mg/kg on three days a week for three months (I). During the second period the dCR dosage was increased to 50mg/kg on five days a week for three months (II). Period III consisted of dCR 25mg/kg and Tetrahydrouridine (THU) 10mg/kg five days a week. The fourth period involved oral guanine hydrochloride in a dose of 10mg - 20 mg/kg/day for three months (IV).

RESULTS

A lymphopenia (1.3x10⁹/1) was already evident at four days of age (Fig.1a). The absolute lymphocyte count rose initially but by three months there was a sustained lymphopenia (0.5-0.8x10⁹/1). This was not altered significantly by any therapeutic regime except during the period of proven Bordetella pertussis infection. Despite initial improvement with dCR alone subsequent values for E rosetting cells were all below the normal range of 50-75% with specific T-cell sub-populations OKT4 (helper) between 21-35% (n=30-50%), OKT8 (suppressor) 4-8%(N=15-30%). OKT6 (thymocyte) sera did not identify any of the peripheral lymphocyte population (Fig.1a). PHA responses, normal at birth, diminished by six months with transient improvement during the pertussis infection and at the commencement of oral guanine (Fig.1b)