Hereditary transcobalamin II deficiency: Clinical findings in a new family

A second family with transcobalamin II (TC II) deficiency was detected. Megaloblastic anemia developed in early life in association with normal serum levels of vitamin B₁₂, but all vitamin B₁₂ was bound to alpha-I-globulin (TC I), and the normal beta-binder (TC II) was lacking. Family studies are compatible with autosomal recessive inheritance. Analogous findings of blocked cellular maturation in the intestine (leading to malnutrition) and in the lymphoid system (agammaglobulinemia) suggest that TC II is of vital importance for rapidly proliferating cells. Complete correction of the clinical manifestations after vitamin B₁₂ therapy in pharmacologic doses was associated with appearance of a B₁₂-binding alpha-2-globulin (fetal binder?).

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In 1971 and 1972 Hakami and associates¹ and Scott and associates² described two siblings with presumably inherited deficiency of transcobalamin II. This is a rather well-defined protein of human serum,³,⁴ which is involved in the transport of vitamin B₁₂ (cobalamin). The authors provided evidence that TC II is necessary for normal cellular maturation of the hematopoietic system. In our observations of a new case, we report an associated severe functional impairment of intestinal mucosa and lymphoid tissue. Repeated administration of large doses of vitamin B₁₂ was followed by rapid improvement of all clinical signs. In this phase most of the vitamin B₁₂ was bound to an alpha-2-globulin.

CASE REPORT

Family B., of Moroccan origin, was referred to us because two sons had died of severe infections in early infancy. The diagnosis of Kostmann's syndrome (infantile genetic agranulocytosis) had been made. The only surviving daughter was apparently healthy. Possibilities of treatment were discussed, if another child to be born should be equally affected. The only hopeful approach appeared to be a bone marrow transplantation, and the possibility of identifying a compatible donor seemed to be fair because the parents were first cousins, and in addition their close relatives presented with multiple consanguinity (Fig. 1). Accordingly, cesarean delivery at the end of the next (uneventful) pregnancy was planned two weeks before the calculated term in order to avoid contamination of the newborn infant. A male infant was transferred immediately into a sterile air-flow unit, where he was nursed during 115 days. No antibiotics were administered, but all cultures of skin, throat, feces, and urine (altogether several hundred) grew no bacteria.

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Abbreviation used
TC II: transcobalamin II

During this time extensive investigations were done: Kostmann's agranulocytosis was excluded, but the diagnosis of agammaglobulinemia was established since the patient was unable to synthesize immunoglobulins and to respond with formation of specific serum antibodies to strong antigenic stimuli. Cellular immune reactions were, however, normal. Therefore, as his agammaglobulinemia was thought to be easily manageable, he was contaminated with an anaerobic intestinal flora, and dismissed home at the age of 3½ months. Three weeks later he was readmitted with a short history of vomiting, anorexia, diarrhea, and upper respiratory infection. Within the next four weeks we observed rapid deterioration with progressive and intractable diarrhea. Severe atrophy of the intestinal mucosa (type 2) and low disaccharidase activity were demonstrated in a jejunal biopsy performed at age 5 months. Malabsorption syndrome was suspected, but no improvement was observed with a gluten-, saccharose-, lactose-, and milk-free diet. In addition the patient developed a severe bronchopneumonia which was treated with gentamycin and immu-
noglobulin therapy. Simultaneously at 7 months of age severe hematologic alterations developed (Fig. 2): a macromegalocytic anemia with very low reticulocyte counts, leukopenia with granulocytopenia, and thrombocytopenia with severe hemorrhagic diathesis. Megaloblastic changes in the erythroid series were present in a bone marrow examination at 5 months of age. Myeloid cells were predominantly immature with vacuolization and giant forms, and megakaryocytes were overseg-

mented. No plasma cells could be found. Pernicious anemia was suspected (Fig. 3).

The patient had received vitamin supplements during the entire time he spent under sterile conditions, including 2 μg vitamin B₁₂ daily, from age 3 weeks to 4 months. At the age of 4 weeks, after the detection of megaloblasts and megalocytes in blood smears, he was given folic acid, first by injection, then by mouth, 15 mg/week to a total of 195 mg (Fig. 2). Both of these
medications were discontinued when he was discharged for the first time at 3½ months of age. At 5½ months of age, 1,000 μg vitamin B₁₂ were administered intramuscularly, and this therapy was repeated three times weekly for the next weeks. A spectacular improvement of the patient, who had been almost moribund, was observed: 1-2 days after the initial dose the boy became cheerful, and within a few days his behavior was completely normal. He developed a reticulocytosis and leukocytosis within 6 days, and his granulocytes increased from 400 to 11,000/mm³ after 17 days. Megalocytic changes disappeared and after 3 weeks the bone marrow contained a completely normal distribution of morphologically normal cells, and there were 0.4% plasma cells.

Four weeks after the first injection of vitamin B₁₂ (6½ months of age), he was discharged in excellent general and nutritional condition. Treatment was continued with 1 mg vitamin B₁₂ intramuscularly once a week and 5 ml gammaglobulin intramuscularly every 10 days, and dietary restrictions were gradually eliminated. The gammaglobulin therapy was discontinued at 9 months of age.

The patient was readmitted at 12 months of age for re-evaluation: reabsorption of 4% of an orally administered dose of radioactive vitamin B₁₂ was noted by the Schilling test. Daily oral administrations of vitamin B₁₂ were substituted for the weekly intramuscular injections. No evidence of B₁₂ deficiency has reappeared in the following 3 months.

Complete absence of transcobalamin II was demonstrated in the patient's serum. After initiation of vitamin B₁₂ therapy a high-binding capacity in the alpha-2 region was found, and the possibility of the presence of "transcobalamin III" or a fetal vitamin B₁₂-binding globulin was entertained.⁵

In the following months the patient developed increasing concentrations of serum immunoglobulins: during therapy with intramuscular gammaglobulin (IgG) at 9 months of age normal serum concentrations of IgM and IgA were measured. Two months after interruption of the gammaglobulin therapy, specific antibodies were detected against antigens which had been injected during the agammaglobulinemic phase while the patient was nursed under sterile conditions (Table II).⁶ These observations document the normalization of antibody formation by the patient.

**FAMILY STUDY (PEDIGREE, FIG. 1)**

In nine relatives of the patient determinations of serum concentrations of transcobalamin II were performed. The parents and three of their relatives had significantly reduced serum levels, whereas the patient's sister and two uncles had normal values. It seems highly probable that the trait is inherited as an autosomal recessive and that heterozygotes can be identified accurately.
Table I. Serum vitamin $B_{12}$ concentrations and $B_{12}$-binding capacity (BBC) in pg/ml

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before $B_{12}$ treatment</th>
<th>After treatment</th>
<th>Mother</th>
<th>Father</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_{12}$ concentration</td>
<td>400</td>
<td>512.0</td>
<td>430</td>
<td>731</td>
<td>200-900</td>
</tr>
<tr>
<td>Unsaturated BBC</td>
<td>2</td>
<td>6.8</td>
<td>315</td>
<td>160</td>
<td>670</td>
</tr>
<tr>
<td>Total BBC</td>
<td>402</td>
<td>518.8</td>
<td>745</td>
<td>891</td>
<td>870-1,570</td>
</tr>
</tbody>
</table>

Table II. Specific immunization and antibody formation

<table>
<thead>
<tr>
<th></th>
<th>1972</th>
<th>1973</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nov. 21</td>
<td>Dec. 1</td>
</tr>
<tr>
<td>Immunization</td>
<td>DiTe</td>
<td>DiTe</td>
</tr>
<tr>
<td>Antibody titer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>diphtheria</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Titer 0 means < 0.06 IU/ml.

**METHODS**

Sterile nursing in a laminar air-flow unit was performed as previously described.7

Bacteriologic controls of sterility were regularly performed.8 The intestinal tract of the infant was contaminated with an anaerobic flora “CRF,” provided by Dr. Van der Waay (Rijswijk) and with an *Escherichia coli* 083 strain provided by Dr. Lodinova (Prague).9

Hematologic studies were performed by routine methods. Studies of leukocyte function were performed with the following methods: Adrenalin test for mobilization of the marginal pool,10 nitroblue tetrazolium test without,11 and with phagocytosis,12 and bacterial killing test.13

Vitamin $B_{12}$ levels in blood were determined by a bioassay and by a radioisotopic assay using hemoglobin-coated charcoal14; unsaturated vitamin $B_{12}$-binding capacity of serum with a modification of this method.15 The $B_{12}$ plasma binders were measured with polyacrylamide gel electrophoresis,16 and qualitatively characterized with special immunodiffusion methods using specific anti TC II antiserum generously provided by Dr. C. A. Hall (Albany).5 Serum concentrations of folate acid were measured by a bio-assay.

Immunologic studies were as follows: antibody-mediated functions were evaluated by measuring immunoglobulins (radial immunodiffusion17 for IgM, IgG, and IgA and radioimmunoassay for IgE and secretory IgA18). Specific antibodies against diphtheria, tetanus, *Haemophilus influenzae* B, pertussis, and key-hole limpet hemocyanin were determined with the hemagglutination inhibition method19-22 after repeated immunizations with the corresponding antigens.

Cell-mediated immunity reactions were measured by in vivo skin testing with specific antigens,23 and especially with key-hole limpet hemocyanin. In vitro studies included phytohemagglutinin stimulation of lymphocytes and mixed leukocyte cultures.

Lymphocyte surface markers were determined with specific immunofluorescent sera for B cells and T cells by the sheep red blood cell rosette method as previously described.24

Intestinal biopsies from the duodenum and the first jejunal loop were performed through a peroral pediatric Watson biopsy tubing.

**RESULTS**

The hematologic data are summarized in Fig. 2. Morphology of the vitamin $B_{12}$-deficient bone marrow elements is illustrated in Fig. 3.

Vitamin $B_{12}$ levels in serum were always within normal range (Table I); serum concentrations of folic acid were slightly elevated. There was virtually no free vitamin $B_{12}$-binding capacity before treatment, but after treating the patient, values were greatly increased. Absence of TC II in the patient was demonstrated by distribution of the binding capacity as quantitatively measured with polyacrylamide gel electrophoresis, and this was confirmed by immunologic determinations using specific anti-TC II anti-serum, as described elsewhere.5

Studies of leukocytic function (adrenalin test, nitroblue tetrazolium test, phagocytic-killing test) were done in the first weeks of life, and were found normal.

Immunologic data were as follows: serum immunoglobulin concentrations were measured repeatedly (Fig. 4); a late appearance of serum IgM was noted which is the first evidence of the patient's own immunoglobulin synthesis. A sharp increase in values was observed shortly after the beginning of vitamin $B_{12}$ therapy. The development of antibodies and the rela-
**DISCUSSION**

Hereditary transcobalamin II deficiency was initially described by Hakami and associates and Scott and associates in two sisters. The clinical features with failure to thrive, diarrhea, atrophy of the mucosa of the tongue, and the hematologic findings of progressive anemia with depression of reticulocytes, leukocytes and thrombocytes, and the typical morphology of macrocytic red blood cells and over-segmented neutrophils are suggestive of the diagnosis of pernicious anemia. Surprisingly, however, the serum vitamin B$_{12}$ level is normal. This apparent discrepancy can only be explained by a disturbed distribution of vitamin B$_{12}$ within the body. Moreover the early appearance of clinical and laboratory signs within the first weeks of life excludes an exogenous deficiency of vitamin B$_{12}$, since the stores in the liver of a newborn infant are sufficient for his needs for at least one year.

Protein TC II is present in serum in very low concentrations of approximately 25 µg/l and the laboratory methods for determination are quite accurate. We found no trace of the normal beta-peak corresponding to TC II in the patient's serum, and intermediate values in the presumably heterozygous carriers of the disease. (Dr. Hakami has confirmed these findings in our patient in his laboratory.) We could further demonstrate the absence of TC II by using a specific antiserum anti-TC II in different immunodiffusion arrangements.

Two important new clinical signs in our patient include severe malabsorption due to atrophy of the small intestinal mucosa, and inability to form specific antibodies and plasma cells. It is probable that the appearance of the full-blown clinical picture in our patient was delayed by two factors: the sterile environment...
During the first 3 months of life, and the continuous administration of small amounts of vitamin B₁₂ (2 µg daily) and of large doses of folate acid (15 mg weekly intramuscularly), the usual vitamin supplements given to our patients reared under sterile conditions. The reduced antigenic stimulation from a bacteria-free environment was replaced by multiple injections of specific antigens (tetanus and diphtheria toxoid etc.). These antigenic stimuli made undoubtedly an impression on the memory cells of the immunologic system, but during the vitamin B₁₂ deficient phase no serum antibodies could be identified, although our agammaglobulinemic patient had normal B lymphocytes. Similar patients have been described by Cooper and Lawton, but are regarded exceptional.

It would appear in our patient that the differentiation of B lymphocytes was already complete before birth, with the infant's vitamin B₁₂ needs provided by translacental transfer from the mother using his own fetal binder, or the differentiation proceeded independently of the availability of vitamin B₁₂. Multiple injections of antigens led to differentiation of antigen-specific memory cells, but the subsequent clonal expansion, maturation into plasma cells, and synthesis of free antibodies were not possible. When sufficient amounts of vitamin B₁₂ were provided during therapy, with maturation of plasma cells specific serum antibody was detected without further immunization.

On the base of this interpretation, it seems likely that the three most rapidly replicating cell systems (hematopoietic tissue, intestinal mucosa, and lymphoid cells) are all dependent on a continuous supply of vitamin B₁₂.

The disease has important therapeutic implications, since control is possible by repeated administration of pharmacologic doses of vitamin B₁₂. During this treatment a new, unusual B₁₂ binder with electrophoretic mobility of an alpha-2-globulin appeared in the serum, a property shared with the "fetal B₁₂ binder." If these two B₁₂-binding proteins turn out to be identical, a property shared with the "fetal B₁₂ binder," two vitally important functions were restituted in our patient after intramuscular or oral vitamin B₁₂ treatment in very high doses, but the mechanism is not clearly understood.

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