Congenital Transcobalamin II Deficiency Presenting Atypically With a Low Serum Cobalamin Level: Studies Demonstrating the Coexistence of a Circulating Transcobalamin I (R Binder) Complex

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A case of transcobalamin II deficiency with several unique features is described. The clinical presentation was typical, except for a slightly delayed age at presentation and the occurrence of apparent neurologic dysfunction from the beginning. The unusual biochemical feature was a low serum cobalamin level (97 pg/ml). Several cobalamin-binding protein abnormalities coexisted and antedated cobalamin therapy. Chief among these was the complexing of all serum R binder (transcobalamin I), leaving the patient with no detectable R binder. This defect appeared to be transient. Noteworthy, too, was a prominent binder of 70,000 mol wt that also carried the bulk of his serum cobalamin after therapy; it was prominent in his presumably heterozygous relatives too. The interrelationship between all these abnormalities is intriguing but unclear. The abnormality in transcobalamin II deficiency is clearly not limited solely to deficiency of transcobalamin II. It is also evident that this entity must now be considered in the differential diagnosis of low serum cobalamin levels in infancy.

CASE REPORT

J.W., a black boy weighing 2.2 kg, was born in March 1981, apparently at term. This was the mother's first pregnancy, and the marriage was not consanguineous. The pregnancy had been complicated only by an episode of urinary tract infection that was treated with antibiotics. The mother's cobalamin intake was adequate. The child was not breast-fed, but was maintained on various formula supplements.

The baby was admitted to Children's Hospital of Michigan in July 1981 with a history of vomiting, diarrhea, and two previous hospitalizations elsewhere. He had chronic oral thrush that was refractory to standard therapy. At 2 mo of age, his blood count had shown Hb 9.4 g/dl, MCV 86 fl, and WBC 10,300/µl, with 38% neutrophils. The blood smear revealed poikilocytes, occasional macrocytes, and adequate numbers of platelets. At 3 mo of age, Hb was 7.5 g/dl, reticulocyte count 0.4%, and WBC 4,000/µl. At that point, iron and vitamin supplements were started, but he was obviously failing to thrive.

Physical examination on admission revealed a small undernourished child with weight and length below the third percentile for age (but normal head circumference), extensive oral thrush, red, excoriated gums, a weak, high-pitched cry, poor head control, and inability to follow a moving light.

Hb was 5.8 g/dl, MCV 89 fl, reticulocyte count 0.1%, WBC 3,400/µl, with 15% neutrophils, and platelets 24,000/µl. Peripheral blood smear showed anisocytosis, poikilocytosis, and occasional macroovalocytes, but no hypersegmented neutrophil nuclei. The bone marrow aspirate displayed severe megaloblastic changes, a 5:1 myeloid:erythroid ratio, and decreased megakaryocyte numbers.

The patient was treated with 50 µg folic acid intravenously, without hematologic response. Red cell and platelet transfusions were given. At this point, pretreatment elevated serum folate (32 ng/ml; normal 4-6) and normal cobalamin levels (500 pg/ml; normal 200-850; see Results for further data) were reported by the commercial laboratory. Urinary methylmalonic acid was not detectable (detection limits 10 µg/mg creatinine). Amino acid screens with nitroprusside and by high-voltage electrophoresis and determination of orotic acid excretion were within normal limits. Transcobalamin II deficiency was suspected and, while tests were pending, the patient was given 1,000 µg cyanocobalamin intramuscularly. A pneumonia developed in the interval. A second cyanocobalamin injection was followed by a brisk leukocytosis, reaching 71,800/µl, normalization of platelet count (peaking at 1,105,000/µl), and reticulocytosis. Along with the hematologic improvement, there...
appeared to be a quick neurologic response, as he began smiling, holding his head up, and responding better to stimuli. However, an aspiration pneumonia held back some of the clinical improvement. Pneumonia progressed despite methicillin and gentamycin therapy, but resolved after treatment with trimethoprim-sulfamethoxazole and erythromycin. Tracheal aspirate had not shown *Pneumocystis carinii*. When the diagnosis of transcobalamin II deficiency was established, therapy was changed from cyanocobalamin to hydroxycobalamin, 1,000 µg every other day.

Serum immunoglobulin levels a week after cyanocobalamin therapy had been started showed IgG of 128 mg/dl (normal for age, 147–988), IgA 36 mg/dl (normal 5–90), and IgM 97 mg/dl (normal 20–118). The following week, IgG was 333 mg/dl, IgA 54 mg/dl, and IgM 680 mg/dl. Further immunologic and leukocyte studies showed elevated white blood count lymphocytes; response to phytohemagglutinin, pokeweed mitogen, and concanavalin A; lymphocyte response in vitro to *Candida*; mixed lymphocyte culture response using unrelated donor lymphocytes; unstimulated nitroblue tetrazolium test (33% positive neutrophils); and C3, C4, and C5 levels.

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In April 1982, he failed to receive any cobalamin injections for 3 wk during a family vacation. Upon his return, Hb was 11.2 g/dl and MCV 71 fl. His peripheral blood showed no megaloblastic changes. IgG was 432 mg/dl (normal for age, 369–1,204), IgA 57 mg/dl (normal 15–118), and IgM 36 mg/dl (normal 39–224). Hydroxycobalamin therapy was restarted on a schedule of 1,000 µg intramuscularly 2–3 times weekly.

MATERIALS AND METHODS

Serum was frozen following centrifugation and thawed for study. Cobalamin content was assayed by radioassay using R binder and by a modification of the method of Kolhouse et al. using pure intrinsic factor as the binder. Unsaturated cobalamin-binding capacity was fractionated and quantitated by Sephadex G200 gel chromatography, as before. Immunologic identification of gel eluate fractions collected into polypropylene tubes was done by incubating them with antisera for an hour at room temperature and rechromatographing. A shift in elution thereupon to the void volume indicated a positive reaction with the antisemur. When the fraction to be tested was itself eluted originally near the void volume on Sephadex gel, rechromatography was done on Sepharose 6B gel columns. The radioactive cyanocobalamin (37Co-Cbl) used was of 220 µCi/µg specific activity (Amersham-Searle Corp., Arlington Heights, IL) when studying small amounts of binding protein. Otherwise, 37Co-Cbl of 10–15 µCi/µg was used. The anti-R binder and antitranscobalamin II antisera were prepared in rabbits and were monospecific for the binder in question. Rabbit anti-human IgG (Fc fragment), anti-IgA, anti-IgM, and anti-λ antisera were obtained from DAKO-immunoglobulins, Copenhagen, Denmark.

Distribution of endogenous cobalamin among the serum cobalamin-binding proteins was determined following Sephadex G200 gel filtration of 0.1–0.4-ml aliquots of untreated serum. Paired neighboring elution fractions were pooled, acidified, and assayed for cobalamin content by radioassay. To confirm the immunologic nature of the binders carrying this endogenous cobalamin, some serum aliquots were also preincubated with antitranscobalamin II or anti-R binder antisemur. The result of endogenous cobalamin distribution was then compared to that of each antisemur filtered alone. Antitranscobalamin II antisemur, being usually diluted 1:100 before use, had negligible cobalamin content, but anti-R binder antisemur, which was used undiluted, had a sizable cobalamin content that had to be subtracted from the pattern obtained.

Second-antibody radioimmunoassays for R binder and for transcobalamin II were developed in order to detect immunoreactive binder molecules even if they were incapable of binding 37Co-Cbl. Both these assays were identical in principle and in virtually all details to the intrinsic factor radioimmunoassay previously described. Details of the R binder radioimmunoassay are described elsewhere. The transcobalamin II radioimmunoassay used a serum containing 5,100 µg/ml total (saturated and unsaturated) cobalamin-binding capacity, 95% of which was transcobalamin II, as the antigen. This antigen was radiolabeled with 37Co-Cbl of 220 µCi/µg specific activity and diluted 1:100 in the assay buffer. Serial dilutions of unlabeled serum of known transcobalamin II content provided the standard curve. Rabbit antitranscobalamin II antisemur was diluted 1:360,000 for use in the assay. All other components, including the assay buffer, and the protocol were identical to those described for the second-antibody radioimmunoassay for intrinsic factor. All specimens were tested in two or three dilutions to assure that at least one point fell within the most sensitive part of the standard curve, usually at 15%–30% precipitation of the radiolabeled antigen. Maximal precipitation was 40%–45% of the radiolabeled antigen, and nonspecific precipitation (tested by supernatant controls of the standard curve and of all test specimens) was 1.6%–2.3%. Absence of transcobalamin II in a specimen was indicated when precipitation of radioactivity equaled the maximal precipitation, without any incremental changes occurring at any dilution of that specimen. Small concentrations below 5 µg/ml could be detected by the slight changes with different dilutions, but could not be accurately quantitated.

The effect of the patient’s serum on other binders was measured in two ways. Effect on holo-binder (binding protein carrying cobalamin) was determined by incubating the patient’s serum with an equal volume of 37Co-Cbl-labeled R binder (from normal human saliva, diluted 1:10) or 37Co-Cbl-labeled transcobalamin II (undiluted human serum, 95% of the cobalamin-binding capacity of which consisted of transcobalamin II) for 1 hr at room temperature. The mixture was then filtered on Sephadex gel to assess for formation of a complex. Effect on apo-binder (binding protein not saturated with cobalamin) was determined by similarly incubating the patient’s serum with a serum containing 35% of its unsaturated cobalamin-binding as transcobalamin I and 60% as transcobalamin II. The mixture was then exposed to excess 37Co-Cbl for 30 min to allow binding to occur and was filtered on Sephadex gel. The resultant chromatographic pattern was compared to that of the serum substrate alone to determine if the patient’s serum had inhibited the ability of either transcobalamin to bind cobalamin or had shifted either peak to the void volume.

The ability of serum to promote cobalamin uptake was tested in a reticuloocyte-rich preparation. Gastric juice intrinsic factor activity was assessed by radioassay and by the ability to enhance 37Co-Cbl
uptake by guinea pig ileal homogenate. Its cobalamin-binding capacity was also fractionated by Sephadex G200 gel chromatography.

RESULTS

Serum (Table 1)

The patient had a low serum cobalamin of 97 pg/ml. We could not ascertain why the commercial laboratory had found a normal level of 500 pg/ml approximately 1 wk earlier. Both sera were obtained before any known cobalamin therapy was given. The low result by our radioassay using R binder was confirmed by microbiologic assay using Euglena gracilis (performed in the laboratory of Dr. Charles Hall, VA Medical Center, Albany, NY) and by radioassay using pure intrinsic factor, the levels being 127 and 96 pg/ml, respectively. An inhibitor suppressing normal cobalamin levels in radioassay was ruled out by mixing experiments in which the patient’s serum did not depress the cobalamin level of a control serum. With therapy, cobalamin levels rose and remained greatly elevated, even when he had not received any injection for 2 wk.

Analysis of his binding proteins revealed several abnormalities. Foremost among these was the total absence of transcobalamin II by chromatographic assay in all three of his serum specimens. This was true for apo-transcobalamin II (Fig. 1) and holo-transcobalamin II (Figs. 2 and 3), although considerable overlap from another large peak appears in Fig. 3. Moreover, none of the other apo- or holo-binders present in his serum reacted with antitranscobalamin II antisera. Second-antibody radioimmunoassay detected no transcobalamin II in his first serum specimen, and only 16 pg/ml was measured in his final specimen. Consonant with this deficiency of transcobalamin II, neither of these two sera promoted cobalamin uptake by reticulocytes (Table 2).

The second abnormality noted was the near total lack of R binder in his serum (Table 1). Chromatography revealed little or no apo-R binder (Fig. 1) and no endogenous cobalamin on R binder in his first two serum specimens (Figs. 2 and 3). Only his last serum had some endogenous cobalamin attached to R binder and had detectable R binder by second-antibody radioimmunoassay.

![Fig. 1. Fractionation by Sephadex gel chromatography of unsaturated cobalamin-binding capacity of J.W. serum from 7/81. (V0, void volume; R, elution position of normal R binder; TC II, elution position of transcobalamin II.) The most noteworthy findings are the lack of a transcobalamin II peak (normal serum produces a transcobalamin II peak that would be off the scale in this figure), and the presence of the peak between V0 and R. The “free cobalamin” peak represents simply the excess 57Co-Cbl that had been added to the serum but not bound.](image-url)
Instead of freely detectable R binder, his sera contained complexed apo-R binder, which was evident on gel chromatography (Fig. 1). The complex eluted between the void volume and R binder fractions. Several lines of evidence showed that this complex, of a molecular weight (mol wt) of approximately 300,000 by Sephadex gel filtration, contained R binder. It reacted with anti-R binder antiserum, which shifted this complex to the void volume on Sephadex G200 gel chromatography, but not with antitranscobalamin II antiserum. The patient's serum from 7/81 also proved capable, upon incubation, of shifting normal salivary holo-R binder (labeled with 57Co-Cbl), but not normal serum holo-transcobalamin II, to the elution position of the complex upon gel chromatography. Moreover, the complex was gradually replaced by free R binder upon repeated thawing and refreezing of the serum specimen with use.

Second-antibody radioimmunoassay for R binder content provided further evidence. The patient's serum from 7/81 by itself precipitated 18.4% of the 57Co-Cbl-labeled R binder antigen in the supernatant control tubes lacking added anti-R antiserum (Table 3). The patient's serum also produced unreadable R binder levels in this assay, because precipitation with appropriate added anti-R binder antiserum exceeded the "maximal precipitation" of the assay system. Table 3 demonstrates the lability of this activity in the patient's serum, as the precipitation in the supernatant

Fig. 2. Fractionation by Sephadex gel chromatography of the endogenous cobalamin in J.W. pretreatment serum (7/81). Note that the amounts of cobalamin shown are very small.

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control tubes declined upon re assay of the same serum. In his later sera, this complexing activity was absent from the outset by both this radioassay and by chromatography.

The complex' specificity for R binder as a substrate was confirmed by the fact that the patient's serum failed to spontaneously precipitate transcobalamin II antigen in supernatant control tubes of the second-antibody radioimmunoassay for transcobalamin II, unlike its behavior in the R binder radioimmunoassay. His sera from 7/81 and 5/82 each precipitated only 2.2%, compared to his mother's serum precipitating 2.1% and control sera 1.6%–2.3%, in the transcobalamin II radioimmunoassay.

The data in Table 3 suggest that the complexing activity was due to immunoglobulin. Omission of the burro anti-rabbit immunoglobulin (second antibody) antiserum from the incubation mixture decreased the precipitation of antigen in supernatant control tubes. However, we were unable to definitively establish its immunoglobulin nature, as incubation of the patient's own $^{55}$Co-Cbl-labeled complex with anti-IgG, anti-IgA, anti-κ chain, and anti-λ chain antisera failed to shift the complex on subsequent gel chromatography. Reactivity may have been compromised at this point, however, by the relative lability of the complex to manipulation.

The final peculiarity of our patient's serum was his prominent binding protein of apparent 70,000 mol wt by Sephadex G200 gel elution between the R binder and transcobalamin II positions. This protein not only constituted a prominent component of his unsaturated cobalamin-binding capacity (Fig. 1) but predominated by far in carrying his endogenous cobalamin, particularly after therapy, when high serum cobalamin levels were achieved (Fig. 3). The nature of this binder is unknown. It reacted neither with anti-R antiserum nor with antitranscobalamin II antiserum, which failed to shift the peak upon subsequent gel chromatography.

Attention may be paid to still another binding fraction, eluting with the void volume (Fig. 1). It provided some unsaturated cobalamin-binding capacity, as seen in virtually everyone's serum. It also seemed to carry some endogenous cobalamin (Figs. 2 and 3), but the proximity of the previously mentioned R binder complex to the void volume fraction made differentiation of these two peaks tenuous in his earlier sera.

Other Fluids

The patient's basal gastric juice, obtained in 2/82, had a pH of 2.0, had normal intrinsic factor concentration (50.72 ng/ml by blocking antibody assay), and effectively enhanced $^{55}$Co-Cbl uptake by guinea pig ileal homogenate. The gastric juice also contained a normal amount of uncomplexed R binder by Sephadex gel chromatography (63.4 ng/ml). Second-antibody radioimmunoassay gave an R binder concentration of 90.0 ng/ml; no complexing activity was detected in the supernatant control tubes of this assay. It should be noted, however, that by this time, the complex was no longer detectable in the child's serum.

A urine specimen was also obtained in 2/82, at a time when the patient was being intensively treated; at that time he had very high serum cobalamin levels, some of which circulated as free cobalamin. The urine contained large amounts of cobalamin (68,305 pg/ml). Virtually all of it was free cobalamin, as determined by assay of gel chromatography eluate fractions.

Family Studies

Only the mother and maternal grandfather were available for study. Both had normal blood counts except for minimal microcytosis (MCV of 80 and 78 fl, respectively, with normal serum ferritin) and mild neutropenia (32% neutrophils of 3,200 WBC/μl and 48% of 3,700 WBC/μl, respectively). Table 1 shows that both had low-normal transcobalamin II levels. Both sera enhanced cobalamin uptake by reticulocytes (Table 2). The uptake mediated by the mother's serum seemed to be less than in controls and in the grandfather, but this cannot be stated with certainty. Both mother and grandfather had normal serum R binder levels. Neither had the R binder complex of the type seen in the propositus, as shown by gel chromatography and by the failure of either serum to precipitate antigen in the supernatant control tubes in the second-antibody radioimmunoassay for R binder (Table 3). Both had unusually distinct 70,000 mol wt binding peaks in their sera (Table 1).

DISCUSSION

Our patient displayed two striking features in the blood that prompted this report. The first was his presentation with a subnormal serum cobalamin level. The second was the clear coexistence of several unusual cobalamin-binding protein phenomena in addition to his transcobalamin II deficiency. The only ways the child differed clinically from the typical course were his slightly delayed age at presentation and apparent neurologic dysfunction at the very beginning. His course and findings, including his hypogammaglobulinemia and lack of methylmalonic aciduria, were otherwise quite typical for congenital transcobalamin II deficiency.5,6 (methylmalonic aciduria is usually but not invariably absent). Macrocytosis was not apparent because of his β-thalassemia trait.
TRANSCOBALAMIN II DEFICIENCY

Until now, it had been a consistent characteristic of transcobalamin II deficiency that serum cobalamin levels were always normal. Since endogenous cobalamin is usually carried by R binder and not by transcobalamin II, normal levels are to be expected in this condition. The explanation for the unexpectedly low serum cobalamin level in our patient is not immediately apparent. Assay artifact can be ruled out. We obtained low levels repeatedly on several aliquots of his pretreatment serum with two different radioassays and with the microbiologic assay, and ruled out any inhibitory activity in the assay system. Another transcobalamin II-deficient child seen subsequently with the identical presentation, whose serum cobalamin level was also low (Meyers and Carmel: in preparation), reinforces the validity of the low level that we observed. Moreover, a recent report described a borderline low serum level in a third child. The discrepancy with the normal result obtained a week earlier in the commercial laboratory is difficult to explain. A processing or other laboratory error may have been responsible for the initial result. Alternatively, the patient’s level could have fallen precipitously between the two samplings for various reasons, including, conceivably, even the intervening folate injection and transfusion. Examination of previous reports of transcobalamin II deficiency does not suggest that folate therapy produces subnormal cobalamin levels. However, details are sparse and the possibility bears further investigation. Folate therapy of acquired cobalamin deficiency has been reported to produce some decline in serum cobalamin levels.

Coexisting cobalamin deficiency seems an unlikely explanation for the child’s low cobalamin level. He was not born to a cobalamin-deficient mother, and he secreted normally functioning intrinsic factor. A Schilling test to exclude coexisting malabsorption was not done, because, for reasons still unclear, cobalamin absorption tests are often abnormal in congenital transcobalamin II deficiency. Moreover, malabsorptive disorders rarely produce clinical deficiency so early in life, even when present at birth; it takes longer, sometimes years, to deplete the stores present at birth. It is conceivable that, given the apparently impaired cobalamin absorption in transcobalamin II deficiency, delayed presentation of the illness may allow cobalamin levels to decline. However, such has not been observed in incompletely treated cases seen at a later age.

The most likely explanation is that the low cobalamin level is related to his other serum cobalamin-binding protein abnormalities. Some of these abnormalities have also been described in previous children with transcobalamin II deficiency whose cobalamin levels were normal. However, our patient’s R binder complexing seems an attractive explanation for the unexpectedly low serum cobalamin level, as R binder is the protein usually carrying serum cobalamin. A similar child with low serum cobalamin under study also has this complex (Meyers and Carmel: in preparation). Nevertheless, other children with similar complexes have had normal cobalamin levels, as have adults with apparently acquired R binder complexes.

Whether or not his neurologic dysfunction was attributable to the low serum cobalamin level is, at present, unclear. Children with transcobalamin II deficiency have thus far displayed a surprising lack of neurologic sequelae. The few with such symptoms were usually children treated with folic acid for a long time before the correct diagnosis was made. The early neurologic manifestations in the present child are therefore unusual. However, it should be noted that there were no focal neurologic deficits, and it is conceivable that the severe developmental delay reflected his severe illness rather than primary neurologic dysfunction.

The findings in our patient also strikingly illustrate that transcobalamin II-deficient children manifest serum cobalamin-binding protein abnormalities beyond simple lack of transcobalamin II. Earlier reports suggested that unusual binders accompany transcobalamin II deficiency, and this was explored in greater detail in a recent survey. Some of these patients displayed abnormalities, such as complexed R binder, and one had an “albumin”-sized binder apparent on chromatography. It seemed that such binder abnormalities often tended to arise, or at least to become more prominent, after treatment. Our patient, however, displayed these additional abnormalities from the very beginning, before the picture was complicated by cobalamin therapy. Based on this case, as well as another currently under study and one examined by Hall and Begley, we suggest that such abnormalities are common in the disorder. They are obviously not induced by cobalamin therapy, though it is possible that therapy amplifies them.

Our patient had little or no normal R binder, whether unsaturated (which is regularly diminished in transcobalamin II deficiency) or saturated with cobalamin (which is unusual and seems likely to be related to his low serum cobalamin level). Whatever R binder was present was complexed by an unidentified substance that appeared to react specifically with this binder but was relatively labile. Although it otherwise resembled an immunoglobulin that complexed R binder in several reported adult cases, our patient’s complexing substance did not react with various
antiimmunoglobulin antisera. Others have also found that such a complex did not behave like IgG.25

This complex may have been an acquired reaction to the lack of transcobalamin II, a part of the hereditary loss of transcobalamin II, or a coexisting disorder. Absence of complexing in his later serum specimens also raises the possibility of a transplacentally acquired factor. The mother did not have such activity, however, though she was not studied until several months after delivery. Whether such complexing assists or hinders auxiliary methods of getting cobalamin into cells is not known. Our patient’s serum did not effectively mediate cobalamin uptake by reticulocytes in vitro; on the other hand, it did not inhibit such uptake the way pure R binder preparations did.

A second coexisting abnormality in the patient’s serum was the presence of a binder of 70,000 mol wt, which is not related immunologically to either transcobalamin I or transcobalamin II. Its molecular size by Sephadex gel filtration is that of albumin, but proof of its nature is lacking. This minor binding protein is detectable in small amounts by gel chromatography when normal binders are missing.9,14,26 Whether its detection in such cases reflects increased amounts of this protein or simply unmasking by absence of normal binder is uncertain. Small amounts have occasionally been detected in normal serum,8 suggesting the latter possibility. On the other hand, our patient’s presumably heterozygous relatives had unusually prominent amounts of the binder, suggesting enhanced elaboration of this protein in this disorder, perhaps on a genetically linked basis. The increase of this binder after the patient’s cobalamin therapy, when it became the major carrier of cobalamin in his serum, was also striking. Others have already speculated about a possibly auxiliary transport role for this protein in transcobalamin II deficiency.26

Our observations establish that transcobalamin II deficiency is an entity more complex than merely lack of transcobalamin II. Elucidation of the origins and roles of these coexisting abnormalities, which our patient demonstrated so strikingly from the very first, should be of great value in understanding cobalamin transport. The new finding that cobalamin levels can be low in this disorder also raises several issues. One that is of particular clinical significance is that transcobalamin II deficiency now has to be added to the differential diagnosis list for low serum cobalamin level in infancy.

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Congenital transcobalamin II deficiency presenting atypically with a low serum cobalamin level: studies demonstrating the coexistence of a circulating transcobalamin I (R binder) complex

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