Combined Congenital Deficiencies of Intrinsic Factor and R Binder

By Jacqueline Zittoun, Juliane Léger, Jeanine Marquet, and Ralph Carmel

Coexisting deficiencies of both intrinsic factor (IF) and R binder were identified in an Algerian boy who presented with severe megaloblastic anemia, growth retardation, and neurologic dysfunction with typical features of subacute combined degeneration of the spinal cord. The anemia responded completely to cyanocobalamin and folic acid. IF was absent from gastric juice, but acid secretion and gastric mucosa were normal. R binders were absent from gastric juice as well as from serum, saliva, and polymorpho-nuclear leukocytes. The patient’s father exhibited absence of R binder in his serum with a low serum vitamin B12 level and was asymptomatic. This unique case of simultaneous IF and R binder deficiencies suggests a genetic association between these two functionally and immunologically dissimilar, but structurally close, vitamin B12-binding proteins.

I

NBORN ERRORS of cobalamin (vitamin B12) metabolism have been identified at different steps of its transport and use:\(^1\): defects of absorption due to an absent\(^2\,^3\) or nonfunctional\(^4\,^5\) intrinsic factor (IF); defects of transport related to absent R binder\(^6\) or to absent\(^7\,^8\) or abnormal\(^9\,^{10,11}\) transcobalamin II (TC II); and defects of intracellular metabolism of cobalamin due to impaired synthesis (or retention) of adenosylcobalamin and/or methylcobalamin.\(^12\)

The severity of the clinical and hematological consequences of these congenital disorders varies. While R binder deficiency does not exhibit any hematological findings, congenital absence of IF and TC II produces a severe megaloblastic anemia within the first weeks of life for TC II, and usually before the first decade for IF.

Each of these congenital disorders has occurred independently in individuals so far. We report here a case of combined congenital IF and R binder deficiency documented during the investigation of megaloblastic anemia associated with neurological dysfunction. This case suggests an association in the genetic inheritance of these disorders and of the localization of genes encoding for these proteins.

CASE REPORT

A 14½-year-old Algerian boy was the third child of a consanguineous marriage. The parents were first cousins and healthy. The pedigree of the family is shown in Fig 1. The patient was born in 1970 at term after an uncomplicated gestation and delivery. At 6 years of age, a hemoglobin (Hb) level of approximately 6 g/dL was noted in the Algerian boy and he was treated with blood transfusions every 3 months. In 1983, he was readmitted because of severe anemia. Physical examination revealed marked pallor, icterus, and an enlarged liver and spleen. The laboratory data were as follows: Hb, 2.4 g/dL; MCV, 100 fL; and reticulocytes, 9 x 10\(^9\)/L. WBCs and platelets were normal, and no abnormalities were found on Hb electrophoresis and red cell enzyme assays. Serum iron was increased (1.75 mg/L). Corticosteroids administered for 1 month had no effect. The boy was again treated with blood transfusions every month, and then every week. In addition to his anemia, the patient displayed severe growth retardation.

In 1984, a bone marrow aspirate showed marked dyserythropoiesis with megaloblastic changes. The child was then treated with a single injection of 1,000 μg of cyanocobalamin and 5 mg/d of folic acid orally for several months, which corrected the anemia and macrocytosis (Hb, 14.5 g/dL; MCV, 76 fL). Meanwhile, the patient had developed difficulty in walking, and exhibited an ataxic gait and exaggerated deep tendon reflexes. The exact time of onset of these neurological signs was not certain.

In 1985, he was transferred to Enfants Malades, Paris for investigation of his growth retardation, neurological problems, and anemia. The patient was underdeveloped (growth more than 5 SD and weight more than 3.5 SD below normal mean) with delayed bone maturation. Despite being more than 14 years old, he showed no evidence of puberty. Endocrine function was normal, with no growth hormone deficiency and with normal thyroid function. Gonadotrophins and testosterone levels were commensurate with the delayed puberty. Neurological examination revealed typical features of subacute degeneration of the spinal cord, weakness of all four limbs, loss of vibratory sense, bilaterally positive Babinski signs, exaggerated deep tendon reflexes, and sphincter disturbances. No cerebellar syndrome, mental retardation, or psychiatric problems were detected. Blood counts were normal (Hb, 13.7 g/dL; MCV, 90 fL; WBC, 12 x 10\(^9\)/L; platelets, 600 x 10\(^9\)/L) and the liver and spleen were not enlarged.

Several other members of the family seemed to have hematological problems in addition to growth retardation (Fig 1). One sister, who probably had anemia, died when she was 5 years old, one brother died at the age of 1 year, but details were not available, and marked dyserythropoiesis was found in another brother who presented with a macrocytic anemia that was corrected with folic acid. The mother and father were apparently healthy and neither one had any evident neurological problems. Only the father, however, made himself available for any tests related to cobalamin status. The rest of the family refused all further investigation.

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MATERIALS AND METHODS

Serum vitamin B12 and folic acid levels were determined by microbiological assays, using Lactobacillus Leichmannii and Lactobacillus Casei, respectively. Serum unsaturated B12-binding capacity (UB12BC) was measured after saturation with an excess of 57 Co-Cyanocobalamin (Amersham, UK; specific activity, 180 to 300 μCi/μg) followed by dialysis at 4°C for 24 hours against 0.9% saline. Gastric fluid was collected via a nasogastric tube for 60 minutes, before and after stimulation with subcutaneous pentagastrin (6 μg/kg). Samples were tested for pH and hydrochloric acid content. An aliquot of each sample of gastric juice was quickly neutralized and depepsinized; it was then assayed for UB12BC and IF content using human anti-IF blocking antibody.6 Saliva was obtained in the fasting state by stimulating secretion with chewing gum. Polymorphonuclear neutrophils (PMN) were isolated after collection of the buffy coat and fractionation on a lymphocyte separation medium (Boehringer Mannheim, France). Pure PMN were collected in the pellet and RBCs were removed by hypotonic shock lysis.

UB12BC of saliva and PMN extract was measured as with serum. Serum, gastric juice, saliva, and PMN extract saturated with an excess of 57 Co-Cyanocobalamin were also fractionated by gel filtration on an Ultrogel AcA34 (IBF) 2.6 x 70 cm column. The buffer used was 0.06 mol/L Tris, 1 mol/L NaCl, adjusted to pH 8 with 1 N HCl. The column was first calibrated with albumin, γ globulins, and lysozyme as standards of known molecular weights (MW). R binders in serum and saliva were also measured by radioimmunossay.44

For some experiments, serum, gastric juice, and PMN extract were incubated with anti-R binder antiserum at 37°C for two hours, then fractionated as above. Two anti-R binder antisera were used. These were raised in rabbits injected either with saliva or with a suspension of pure PMN containing only R binders. In another experiment, saliva was incubated with a monoclonal anti-TC II antibody13 and filtered on the column.

RESULTS

In 1985, the serum vitamin B12 level, assayed on two different occasions was 50 and 55 ng/L (normal range, 200 to 500 ng/L). The serum folate level was elevated (29 μg/L), but the patient had received folic acid several days before.

The Schilling test produced a subnormal result (urinary excretion of 0.2% in 24 hours) that was corrected to 11% when repeated with exogenous IF. A D-xylose absorption test was normal.

Gastric biopsies showed normal gastric mucosa on different samples, and intestinal biopsies showed very mild atrophy. Analysis of gastric juice showed normal free hydrochloric acid output, with a total absence of IF on gel filtration. However, because the gastric juice also had undetectable UB12BC, coexisting absence of R binder was suspected and we searched for this protein in other secretions and in PMN.

The patient’s serum had an UB12BC of 1,575 ng/L.

### Table 1. Levels of Vitamin B12 Binders

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient (ng/L)</th>
<th>Patient’s Father</th>
<th>Normal Controls (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (ng/L)</td>
<td>1,575</td>
<td>960</td>
<td>1,000-1,740</td>
</tr>
<tr>
<td>UB12BC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R binder*</td>
<td>Undetectable</td>
<td>Undetectable</td>
<td>280-620</td>
</tr>
<tr>
<td>TC II*</td>
<td>1.455</td>
<td>935</td>
<td>600-1,250</td>
</tr>
<tr>
<td>Peak (MW 75,000)*</td>
<td>120†</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Gastric juice (μg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UB12BC</td>
<td>Undetectable</td>
<td>ND</td>
<td>&gt;40</td>
</tr>
<tr>
<td>R binder*</td>
<td>Undetectable</td>
<td>ND</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Intrinsic factor*</td>
<td>Undetectable</td>
<td>ND</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Saliva (μg/L)</td>
<td>0.25</td>
<td>ND</td>
<td>&gt;25</td>
</tr>
<tr>
<td>UB12BC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R binder*</td>
<td>Undetectable</td>
<td>ND</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Peak (MW 35,000)*</td>
<td>0.25‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN (ng/10^7 cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UB12BC</td>
<td>0.15</td>
<td>ND</td>
<td>2-4</td>
</tr>
<tr>
<td>R binder*</td>
<td>Undetectable</td>
<td>2-4</td>
<td></td>
</tr>
<tr>
<td>Peak (MW 35,000)*</td>
<td>0.15*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*After chromatographic fractionation on ultrogel ACA34.
†Not reacting with anti-human R binder antiserum.
‡Not reacting with anti-human R binder antiserum or with monoclonal anti-TC II.
corresponding to R binder was identified; the bulk of eluted vitamin B12 levels, absence of IF in the gastric juice, normal criteria: macrocytic anemia associated with decreased serum these congenital abnormalities of cobalamin metabolism has been described2'3'6 The diagnosis requires the following both IF and R binders in the same patient. Until now, each of these congenital abnormalities of cobalamin metabolism has occurred separately. Approximately 40 cases of congenital pernicious anemia have been described.2'3'16 The diagnosis requires the following criteria: macrocytic anemia associated with decreased serum vitamin B12 levels, absence of IF in the gastric juice, normal gastric free acid and pepsin, and normal gastric mucosa. Most patients develop anemia in the first few years of life as the prenatal stores of cobalamin become exhausted, but ages at presentation can vary.3,17 Congenital pernicious anemia responds completely to parenteral cobalamin therapy.

DISCUSSION

This is the first identified case of congenital absence of both IF and R binders in the same patient. Until now, each of these congenital abnormalities of cobalamin metabolism has occurred separately. Approximately 40 cases of congenital pernicious anemia have been described.2'3'16 The diagnosis requires the following criteria: macrocytic anemia associated with decreased serum vitamin B12 levels, absence of IF in the gastric juice, normal gastric free acid and pepsin, and normal gastric mucosa. Most patients develop anemia in the first few years of life as the prenatal stores of cobalamin become exhausted, but ages at presentation can vary.3,17 Congenital pernicious anemia responds completely to parenteral cobalamin therapy.

In the case reported here, the 7 years that elapsed between the first signs of anemia and adequate treatment with parenteral vitamin B12 probably produced the neurological dysfunction that was consequently irreversible with hydroxocobalamin. The concomitant R binder deficiency probably did not contribute to the patient's hematological and neurological signs since both are adequately explained by his congenital pernicious anemia.

No hematological consequence has ever been attributed to R binder deficiency, and all four previous cases were inadvertently discovered in adulthood6; some of the patients were even elderly.14,15 Nevertheless, one of the original cases of R binder deficiency6 had an unexplained neurological syndrome that may have been attributable to cobalamin deficiency, but also included features not usually associated with cobalamin defects such as scanning speech and seizures (Carmel, unpublished data). Sigal et al recently described a patient with neurological symptoms and raised the possibility that R binder deficiency produces neurological dysfunction.19 However, that patient was not a true case of R binder deficiency because R binder was lacking only in his plasma.20 In addition, no other R binder-deficient patient has had similar neurologic problems, such as the affected older brother of the previously mentioned neurologically impaired patient and the 54-year-old affected father of the patient reported here. Thus, while we cannot be absolutely certain, it seems unlikely that our patient's neurological difficulties arose from his R binder deficiency.

The only abnormality regularly detected in patients with R binder deficiency is a low serum cobalamin level without any sign of cobalamin deficiency. The absence of R binders in serum, which usually bind most of the endogenous cobalamin (hence the low serum cobalamin level), coincides with an absence of this binder in all other sources expected to contain it (saliva, tears, gastric juice, and PMN). Body cobalamin stores are normal, as shown by normal amounts of cobalamin in the liver of one of these patients, with normal proportions of the several forms of cobalamin.21

In the present patient, R binder deficiency was discovered fortuitously during analysis of gastric juice for IF because the total absence of UB12BC in gastric secretion was unusual. Gastric R binder levels were normal in four cases of
congenital pernicious anemia that we have studied (Zittoun, unpublished data). The absence of R binder in the present patient’s gastric juice was associated with its absence in serum, PMNs, and saliva. A small cobalamin-binding peak eluting on Ultrogel chromatography between R binder and TC II and not reacting with anti-R antiserum was found in the serum of the patient and his R binder-deficient father. Chromatography of PMN extract and saliva exhibited a peak that eluted like TC II, but did not react with either a monoclonal anti-TC II antibody or with anti-R antiserum.

This combined congenital disorder of two functionally and immunologically unrelated cobalamin binders in the same patient raises questions concerning their inheritance and the localization of genetic loci determining the synthesis of these two products.

Failure to elaborate IF is transmitted as a recessive autosomal characteristic. As the patient’s parents were not anemic, they were, at most, carriers of the genetic trait. Other children in the family may have had IF deficiency, since one girl died at the age of 5 years with unexplained pallor and one brother had a macrocytic anemia corrected with folic acid. However, we were unable to carry out the requisite studies to document this.

The first case of congenital deficiency of R binder reported in two brothers suggests an autosomal recessive or possibly even X-linked disease. The father of our young patient had this deficiency, based on the absence of R binder in his serum, even though other sources of this binder were not tested in him.

One can hypothesize that our patient’s congenital pernicious anemia and congenital R binder deficiency were transmitted independently and are merely coincidental. However, the occurrence of two unusual disorders in the same patient makes coincidence unlikely. It is also possible to suggest that the genetic loci for IF and R binders are located on the same chromosome, although extremely close linkage seems unlikely since this is the first report of combined disorders. Some genetic link between the coexisting congenital deficiencies of these two cobalamin-binding glycoproteins is further suggested by the close structural similarity between the two proteins. This similarity suggests a common origin for IF and R binder, and our patient provides clinical support for such a hypothesis. However, the inability to adequately study all other members of the patient’s family prevents definite conclusions about the genetic transmission of these two unusual abnormalities.

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REFERENCES

Combined congenital deficiencies of intrinsic factor and R binder

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