Selective cobalamin malabsorption and the cobalamin-intrinsic factor receptor*

Ralph Gräsbeck

Minerva Foundation Institute for Medical Research, FIN-00250 Helsinki, Finland

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The disease is characterised by cobalamin (Cbl) deficiency in children 0–5 years old, causing failure to thrive, infections, megaloblastic anaemia, neuropathy, and mild general malabsorption; slight proteinuria is common. Cbl injections produce remission, but Cbl malabsorption and proteinuria persist. About 250 cases have been reported. Dogs also have it. The heredity is autosomal and recessive. The physiological and pathological absorption mechanisms are described: Cbl liberated from food by digestion is first bound to haptocorrin, but in the intestine it is transferred to intrinsic factor. In the ileum the complex attaches to a receptor on the enterocytes; this requires neutral pH and Ca^{2+}. The receptor is a membrane-bound glycoprotein consisting of multiple subunits. The receptor-ligand complex is endocytosed and degraded in lysosomes, and the vitamin is transferred to transcobalamin which carries it to tissues. The same receptor is strongly expressed in the kidneys, but urine also contains its activity which can be assayed for diagnosis. The basic lesion is an error in the ileal receptor. In the affected dogs the synthesised receptor is retained intracellularly. Urine and ileal biopsies from human cases contained little receptor but it had conserved affinity for the ligand. Recently examined Arab patients did not excrete reduced amounts of the receptor. Apparently, the disease has subsets, such as different structural errors in the receptor and possibly faulty transport inside the enterocyte. The cause of the proteinuria is unknown but kidney damage due to severe Cbl deficiency and an error in a multiligand renal receptor are among the possibilities.

In the late 1950ies a “new” hereditary disease or syndrome was described, selective vitamin B_{12} (or cobalamin, Cbl) malabsorption (SBMA). The following review summarises what is currently known about the biochemistry of the intestinal absorption of Cbl.

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tel. +358-9-4771003, fax +358-9-4771025

Abbreviations: Cbl, cobalamin (vitamin B_{12}); IF, intrinsic factor; Tc, transcobalamin (transcobalamin 2); SBMA, selective vitamin B_{12} (cobalamin) malabsorption; Hc, haptocorrin (also called R protein or cobalophilin).
(incidentally the first known instance of protein-induced endocytosis) and presents the likely molecular cause of the disease.

**DISCOVERY AND MAIN FEATURES OF SELECTIVE VITAMIN B₁₂ MALABSORPTION (SBMA)**

In 1957, a boy and a young woman with obscure Cbl-responsive anaemia combined with mild proteinuria were examined. They were singularly thoroughly investigated and all known causes of Cbl deficiency eliminated, including pathological intestinal flora, inactive intrinsic factor (IF) and transcobalamin (Te or Te₂) deficiency (as known today). Since the parents of the female were first cousins two ways, the condition was thought to be hereditary. The proteinuria appeared to be benign but kidney biopsies failed to reveal its cause. The condition was shown to be selective vitamin B₁₂ malabsorption [1]. This term is now in general use alone or with the names of the discoverers.

Simultaneously, a Norwegian paediatrician Olga Inerslund published her dissertation “Idiopathic Chronic Megaloblastic Anemia in Children” [2].

Her material consisted of 10 cases from 6 families, 8 with proteinuria. Half of the cases had anatomical anomalies of the urinary tract (double ureters, horseshoe kidney, etc.). Some had unspecific aminoaciduria. The investigations were mostly purely clinical and did not reveal the pathogenetic mechanism, hence the term “idiopathic”. However, malabsorption or a metabolic defect were suggested as causes. Both sexes were affected and the heredity seemed recessive.

The similarity of the two sets of cases suggested that they represented the same disease. However, complete certainty was not reached until in 1963 Inerslund & Björnstad [3] performed absorption tests with radioactive Cbl on the Norwegian patients.

In 1967 we published by invitation a *Europa medica* article [4] which included thoroughly studied new cases, but because of the language barrier it never attracted attention. Microscopy of ileal biopsies and assays of Te’s showed nothing pathological. Absorption studies with oral megadoses of Cbl indicated less absorption than in pernicious anaemia, where a small but therapeutically sufficient fraction is absorbed. An important conclusion was reached: Absorption tests and the final diagnosis should be made after correction of the deficiency state, which often causes mild general malabsorption and the corresponding misdiagnosis.

By 1997 approximately 250 cases had been reported in the literature [5-9]. The criteria used for diagnosis have rarely been as rigid as used by us [1, 4, 6, 7], but most of the reported cases are convincing. The differential diagnosis is relatively easy as there are few closely resembling conditions, essentially only Te deficiency, juvenile pernicious anaemia (absent or inactive IF) and general malabsorption. When diagnosed, the prognosis of SBMA is excellent provided monthly Cbl injections are given.

The age of onset, i.e. appearance of megaloblastic anaemia, has been arbitrarily set at 0-5 years [6, 7], but much later appearance has also been reported [10,11]. Acquired forms may exist [4]. Cases lacking proteinuria have been found from the beginning [2]. Proteinuria was absent in 22% of 36 Turkish cases [5] and in 8 of 10 recently examined Finnish cases ([12], Grösbeck, unpublished). A logical explanation is that severe Cbl deficiency in infancy causes an irreversible kidney lesion and that in recent years early diagnosis and treatment have prevented it from developing. The urinary proteins are of middle and low molecular mass, mostly albumin, indicating glomerular proteinuria [7]. It persists in spite of treatment but kidney function does not deteriorate.

There are reports describing simultaneous occurrence of various anomalies in conjunction with SBMA: urinary tract malformations and aminoaciduria [2], beta-thalassaemia [13], dolichocephaly [14] and chromosomal deletion [15]. These features are not clearly linked to SBMA.

Fyle et al. [16] reported a similar disease in dogs, giant schnauzers. Dogs have physiological proteinuria, which was not increased in the affected animals. Additional cases were produced by inbreeding and biochemi-
cal studies were conducted on intestine and other organs [17].

GENETIC BASIS

Imerslund's conclusion that the inheritance is autosomal and recessive has been repeatedly confirmed [6, 8]. The inheritance is similar in the dogs [16].

Linkage analysis of the combined Finnish-Norwegian material using microsatellite markers assigned the recessive-gene locus for the disease to chromosome 10, its pericentromeric region, probably the short arm. There was no evidence of non-penetrance [6].

BIOCHEMISTRY

Absorption and malabsorption of Cbl

Three Cbl-binding proteins play a central role in the absorption and transport of Cbl: IF, secreted in man by the stomach, haptocorrin (Hc, also called R protein or cobalophilin) found in numerous cells and body fluids, and Tc occurring in blood plasma and semen and synthesised by liver cells and enterocytes. IF and Hc are glycoproteins, whereas Tc lacks carbohydrate. The Cbl-binding site of IF accepts only cobalamin, whereas Tc binds a vast array of corrins most of which lack vitamin activity. Tc is intermediate in this respect [18–20].

Today it is known that Cbl liberated from food by peptic digestion is first bound to Hc, mainly from saliva. Pancreatic enzymes liberate Cbl from this complex. The vitamin is then bound to IF, travels down to the ileum, where the enterocytes on their microvilli have receptors for the Cbl-IF complex; free IF is poorly bound. The attachment to the receptor requires neutral pH and the presence of Ca^{2+} and involves a binding site on IF activated by the binding of Cbl. The complex is endocytosed and IF degraded by lysosomal enzymes. Cbl is then transferred to Tc, which leaves the enterocyte and enters the blood. Tc transports Cbl into tissues provided with the proper receptor, in a way very similar to that of the action of IF [19]. The role of Hc is not altogether clear. It binds corrins with amazing affinity and may prevent antagonistic analogues from entering the body and take the absorbed compounds to the liver for excretion with the bile. However, congenital lack of Hc has no or very weak effects, possibly neurological [19].

Malabsorption of Cbl results from lack of dysfunctioning of one or several of these factors and steps. Many of these possible causes were eliminated early, e.g. defective IF and lack of Tc, which gives megaloblastic anaemia in newborns and malabsorption of Cbl. In early studies, administration of intestinal or pancreatic juice appeared to increase Cbl absorption in some cases of SBMA [21], but others failed to find the same [4]. The current view is that the ileal Cbl-IF receptor is at fault. Ileal biopsies were found to have decreased or absent receptor activity [22, 23] but Mackenzie et al. found no decrease in their patients [24].

The Cbl-IF receptor

The structure of the Cbl-IF receptor has been elucidated by us [25–28], lately cooperating with a French group [29], and by an American team [30]. Pig, rat and dog material has mainly been used, but data on man [26] and opossum [31] have also been published. Recently, the receptor has been found in large quantities in the proximal tubular epithelial cells of the kidney [31, 32]. It has also been detected in liver [33, Gräsbeck, unpublished], placenta, foetal colon and the visceral yolk sack of the embryo [30]. It is not expressed in colon, but it has been found in two colon cancer cell lines, Caco-2 and HT 29 [34, 35]. The expression of the receptor is increased by oestrogens and pregnancy. Brain death decreases it and psychic stress marginally [36]. The intact receptor does not bind Cbl but cobinamide (an “incomplete” Cbl molecule). However, Hc has much greater affinity for this ligand and prevents it from binding to the receptor [37].

The receptor has been solubilised with detergent (Triton X-100) and purified by affinity chromatography. Three types of media have been used: Cbl, IF or anti-IF immunoglobulin covalently coupled to Sepharose [20,
28, 29, 38]. Then Cbl or IF is adsorbed to produce the bound ligand IF-Cbl. The receptor is adsorbed at neutral pH and in the presence of Ca\(^{2+}\) and eluted by lowering the pH or chelating Ca\(^{2+}\). The orientation of the medium-bound ligand is of importance; covalently bound IF gave a better yield than Cbl-Sepharose [29].

The purification products differ, apparently depending on the species and the affinity medium. The "intact" receptor appears to be a large and multimeric molecule (figures over 1 million Da have been published) [38]. In our hands reduction with 2-mercaptoethanol gave two subunits [26-28] (some have found more), one hydrophilic (alpha) and one hydrophobic (beta) which in human material had the molecular masses of 90 kDa and 140 kDa, respectively [26]. Subsequent studies without reduction indicated one peptide chain of approximately 220 kDa [29]. Carbohydrate is present in the receptor molecule [32, 39] and Ca\(^{2+}\) causes aggregation of subunits [40].

The size of the receptor in the ileal brush border has been found to be about 180 kDa, i.e. by 50 kDa smaller than in kidney and the colonic cell lines [30]. Therefore, in vivo the intestinal receptor may be modified by pancreatic proteases. This is known to occur with sucrase-isomaltase and aminopeptidase of apical brush borders and may explain the finding of two subunits [30]. The isolated dog receptor bound human, hog and dog Cbl-IF, but not free Cbl, free IF or Cbl-Hc [38].

The receptor has been solubilised by papain digestion of the intestinal mucosa [27, 41, 42]. The liberated fragment has the capacity to bind Cbl-IF and is hydrophilic, not binding to phenyl-Sepharose [42]. In our hands the porcine fragment had a mass of 45 kDa with a strong tendency to dimerise, but Cbl-IF displaced one monomer [27]. This may be relevant for the function and evolution of the receptor system. The French group used Cbl-Sepharose (without IF) to purify the porcine fragment. It had the mass of 69 kDa and contained 3.8% of O- and N-glycosylated carbohydrates. It bound both Cbl-IF and free Cbl [42]. In the dog, the papain-activated protein represented 85% of the intact receptor (186 kDa vs. 222 kDa), suggesting that only 17% of the receptor is inserted in the cell membrane [41].

Interestingly, the papain fragments characterised by different authors are rather dissimilar.

The American team has studied the receptor in rat kidney cortex and epithelial cells from the proximal tubules [32]. In vitro translation using poly A\(^{+}\) RNA showed that the receptor is synthesised as a single polypeptide of 210–220 kDa. The apical expression of the receptor is slow and occurs with the processing of high mannose linked oligosaccharides to the complex type. It is regulated by the biosynthetic and not the endocytic pathway. The internalised receptor is degraded and not recycled. After synthesis, the molecule folds in order to fit the ligand and its antibody. Microtubule-active drugs such as colchicine decrease the apical expression. The asparagine-linked sugars also influence it. Palmitoylation of some cysteine residues occurs posttranslationally [30, 43].

Studies on the dogs with SBMA indicated that their receptor is present intracellularly but poorly expressed apically. Its susceptibility to proteolysis was increased and it contained high mannose-type N-linked sugars. Thus a trafficking defect was present, probably caused by an alteration in the structure of the receptor [30].

The presence of the receptor in kidney is teleologically puzzling, especially as there is more of it than in the intestine. Our team has found immunoreactive IF in human urine, where we assumed it would come from the gastric mucosa like pepsinogen, by backward (endocrine) secretion [44]. Pernicious anaemia patients excreted less, but unfortunately, urinary anti-IF antibodies mimic IF in radioimmunoassay and preclude the diagnostic use of the test [45]. That the renal receptor catches IF in vivo was demonstrated by dissociating IF from it [46]. However, Nature's arrangement to have in the kidney a special mechanism to salvage a gastric protein seems rather luxurious. It is therefore intellectually gratifying that evidence was recently produced that the IF receptor is identical with a multiligand binder gp280, found in intestine, kidney and yolk sack, and which is the target of teratogenic antibodies.
[47, 48]. Incidentally, a renal Tc binder was also found to be identical with a multiligand receptor, megalin, previously designated gp930 [49].

**Urinary receptor, a diagnostic test**

Because of the presence of immunoreactive IF in urine, we have searched for and found the Cbl-IF receptor in urine. It is hydrophobic enough to bind to phenyl-Sepharose, which was used to separate free and bound ligand (Cbl-IF) and to quantitate the receptor. The activity of the urinary receptor correlated well with that measured on ileal biopsies of the same individuals. In comparison with five controls the urinary receptor activity was clearly low in four patients with SBMA. However, Scatchard analysis indicated that the affinity of the receptor for the ligand was not changed. We concluded that the assay of urinary receptor may be helpful for diagnosis [23, 40].

These studies have now been repeated on 10 Finnish patients, included in the genetic study described above. The findings were similar: low excretion of the receptor with conserved affinity [12].

Similar studies have been conducted on two families in Kuwait, one with 4 affected boys (one deceased and one with late onset, 15 years) and 7 healthy sisters, the other family with 4 affected (1 girl) and 4 healthy sibs. X-Linked inheritance was suspected in the case of the first family [11]. We have now assayed the urines of the Kuwaiti families for receptor activity. There is no decrease of activity or affinity; in the second family the patients may even excrete more than controls (Grasbeck et al., unpublished). Examples of the results of assays of urinary receptor are given in Figs. 1 and 2. Linkage analyses on these and other Arab cases are being performed.

**CONCLUSION**

SBMA is probably the most usual cause of Cbl deficiency in children. It must be suspected in cases where young persons have macrocytic and/or megaloblastic anaemia, pancytopenia or neurological disease. The disease may also appear in the form of recurrent infections or mere failure to thrive [50]. Accompanying proteinuria is strongly suggestive.

The cause of the disease is failure to translocate Cbl from IF in the ileum to Tc in the enterocyte. Mostly, the ileal receptor for IF-Cbl has been implicated, but there are many ways in which it can become absent or malfunctioning. Consequently, there is reason to believe that there are subsets of SBMA, e.g. lack of synthesis of the receptor and failure to transport the synthesised product to the cell surface. The former may be the cause of the Scandinavian cases, as evidenced by almost total absence of the receptor in ileal biopsies and urine. The latter seems to be the

![Figure 1. Urinary Cbl-IF receptor activity.](image)

Scatchard plots of one healthy person, one Finnish and one Kuwaiti patient. Abbreviations: B, bound; F, free cobalamin-intrinsic factor.
However, its common absence and decreasing frequency suggest that the irreversible kidney damage is more probably caused by Cbl deficiency in early infancy.

It is also interesting that in health and in pernicious anaemia (but not in SBMA) the receptor is able to bind free Cbl, notably if present at high concentrations. Especially in view of the role of the multiligand receptors, phenomena and diseases analogous to those described seem likely to occur in the transport of other substances than Cbl.

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REFERENCES


