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Regulated expression of intrinsic factor-cobalamin receptor by rat visceral yolk sac and placental membranes

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Intrinsic factor-cobalamin receptor (IFCR) activity in visceral yolk sac and placental membranes is regulated during pregnancy in rats. While the IFCR activity declined in the visceral yolk sac membranes by 15-fold, it rose nearly 20-fold in the placental membranes from fourteen to nineteen days of gestation. The visceral yolk sac membranes revealed a 230 kDa protein that co-migrated with pure rat renal IFCR. This 230 kDa band was also identified as IFCR in both the membranes by immunoblotting with anti-serum to rat renal IFCR. Immunoprecipitation of ^{35}S labeled proteins obtained from in vitro translation using visceral yolk sac mRNA from 14-day pregnant rats, yielded on SDS-PAGE a single band of 220 kDa, while those obtained from 19-day pregnant rats did not. The binding of intrinsic factor-cyano[^{57}Co]cobalamin complex to the visceral yolk sac membranes was inhibited by preincubation of these membranes with anti-serum to rat IFCR but not with anti-serum to rat asialoglycoprotein receptor or mannose or mannan or *N*-acetylglucosamine. Based on these results, we suggest that the IFCR activity, protein expression and mRNA levels in fetal membranes are regulated during pregnancy and may play an important role in the maternal-fetal transfer of cobalamin.

Introduction

Intrinsic factor-cobalamin receptor (IFCR) is a 200–230 kDa integral membrane protein that is expressed in the apical brush-border membranes of the intestine [1] and kidney [2]. IFCR promotes the uptake of cobalamin (Cbl; vitamin B-12) bound to gastric intrinsic factor (IF). In both these tissues IFCR activity is regulated during post natal development [3,4] and the peak IFCR activity is noted around the 20–24th day. Although IF mediated absorption of Cbl occurs in the adult intestine [5] recent studies have demonstrated transcytosis of Cbl bound to IF and IFCR expression in colon adenocarcinoma cells [6–8] that have characteristics of fetal enterocytes. Furthermore, one recent study [9] has demonstrated IFCR activity in the fetal intestine and colon. These studies have suggested that IFCR expression may also be a property of fetal cells. In order to look into this possibility we have examined IFCR expression in visceral yolk sac, which like the intestine and kidney is derived from mesoderm and in other fetal derived membranes like the placental membranes. The results of the present study shows that IFCR activity is expressed in both the visceral yolk sac and placental membranes, but the activity in these membranes is regulated differently during the early and late gestation periods.

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Material and Methods

Timed pregnant rats purchased from Sasco (Omaha, NE) were anesthetized and decapitated on day 14 and day 19 of gestation. The placenta and the visceral yolk sac were dissected and collected separately according to Muller et al. [10]. The tissues were washed with ice-cold phosphate-buffered saline to remove blood and were homogenized in 4 volumes (w/v) 10 mM Tris-HCl (pH 5.0) containing 0.1 mM phenylmethylsulfonyl fluoride, 140 mM NaCl and 5 mM EDTA. Total membranes were obtained by centrifugation at $20000 \times g$ for 30 min. The sedimented membranes were suspended and homogenized in the same buffer but pH 7.4 and without EDTA and used for the Ca^{2+} specific binding of IF-[^{57}Co]Cbl as described earlier [11]. In some experiments, prior to the addition of IF-[^{57}Co]Cbl (3.7 pmol), the visceral yolk sac membranes (250–500 μg protein) were incubated with anti-serum to rat renal IFCR or rat hepatic asialoglycoprotein receptor or with various sugars. Rat gastric IF,

anti-serum to rat renal IFCR used in these studies were prepared according to Seetharam et al. [12] and Seetharam et al. [2]. Anti-serum to major (RHL1) and minor (RHL 2 and 3) subunits of rat liver asialogylcoprotein receptor was a gift from Dr. Ann L. Hubbard (Johns Hopkins University, Baltimore).

SDS-PAGE electrophoresis using pure rat renal IFCR (2 μg) or total visceral yolk sac and placental membranes (100 μg protein) were carried out on 5% gels according to Laemmli [13]. Western blots using anti-serum to rat renal IFCR and ^{125}I-protein A were carried out as described earlier [2].

Total RNA from freshly dissected visceral yolk sac from 14-day and 19-day pregnant rats was isolated by the guanidine thiocyanate method of Chomczynski and Sacchi [14]. Poly(A$^+$)RNA was purified by chromatography using oligo(dT) cellulose [15]. Cell free translation using poly(A$^+$)RNA (5 μg) and reticulocyte lysate system (Promega Biotech, Madison, WI), was carried out essentially as described earlier [16]. Immunoprecipitation of the ^{35}S-labeled products was carried out using 2–5 μl of antiserum to rat kidney IFCR or preabsorbed IFCR antiserum.

Results

Specificity of IF-[^{57}Co]Cbl binding

Significant amount of IFCR activity is expressed in the visceral yolk sac and placental membranes from 14 and 19 day gestation rats (Fig. 1). The IFCR activity in the visceral yolk sac membranes declined from 1200 fmol/mg protein to 81 fmol/mg protein during day 14 to day 19 of gestation, while it rose in the placental membranes during the same period from 24 to 476 fmol/mg protein. In order to confirm that the Ca^{2+}-dependent binding of rat IF-[^{57}Co]Cbl to visceral yolk sac and placental membranes was due to specific binding to IFCR, the binding of IF-[^{57}Co]Cbl was carried out following preincubation of these membranes with anti-serum to pure rat kidney IFCR, pure rat liver asialoglycoportein receptor and various monosaccharides. Anti-serum to rat renal IFCR blocked the binding of IF-[^{57}Co]Cbl to visceral yolk sac membranes (Fig. 1: 1,2,3). Anti-serum to rat hepatic asialoglycoprotein receptor, which is known to bind [^{57}Co]Cbl complexed to haptocorrin, another Cbl binding protein, was not able to block the binding of IF-[^{57}Co]Cbl to the visceral yolk sac membranes (Fig. 1: 4,5,6). Furthermore, the binding of rat IF-[^{57}Co]Cbl to the visceral yolk sac membranes was not blocked by sugars such as mannose, mannan and *N*-acetylglucosamine (Fig. 1: 7,8,9). Similar results were also noted with the binding of IF-[^{57}Co]Cbl to the placental membranes that were preincubated with these reagents (data not shown).

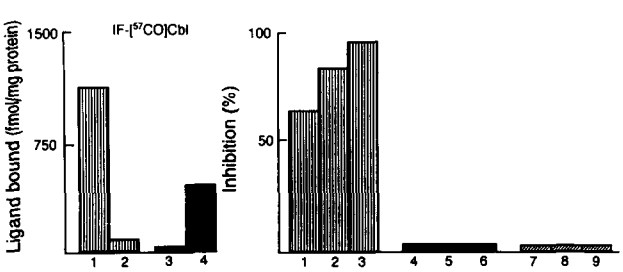

Fig. 1. IFCR activity in the rat visceral yolk sac and placenta. (Left) Rat IF-[^{57}Co]Cbl (3.7 pmol) was used to determine the Ca^{2+}-dependent binding to the total membranes (left) obtained from visceral yolk sac (▥) or placental (■) membranes from rats of 14 day (1, 3) and 19 day (2, 4) gestation. (Right) The binding of rat IF-[^{57}Co]Cbl to the visceral yolk sac membranes (14-day-old) were carried out following an initial preincubation of the membranes with 2 μl (1 and 4), 5 μl (2 and 4) and 10 μl (3 and 6) of anti-serum to rat IFCR (1, 2, 3) or asialoglycoprotein receptor (4, 5, 6) or with 2 mM mannose (7), 5 mg mannan (8) or 100 mM *N*-acetylglucosamine (9).

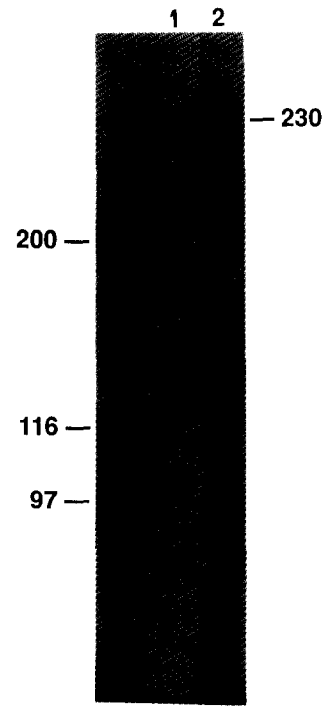

Fig. 2. SDS-PAGE of visceral yolk sac membrane proteins. Visceral yolk sac membrane protein (100 μg, 2) and pure rat renal IFCR (2 μg, 1) was separated on SDS-PAGE (5%) and the bands visualized by Coomassie blue staining.

SDS-PAGE analysis and immunoblotting with anti-serum to IFCR

SDS-PAGE analysis (Fig. 2) of visceral yolk sac membranes revealed a 230 kDa protein band that co-migrated with the pure rat renal IFCR. Further confirmation of the 230 kDa band as IFCR was obtained by immunoblotting studies. When the visceral yolk sac and the placental membrane proteins (100 μg) were separated on SDS-PAGE (5%) and probed with anti-serum to adult rat kidney IFCR and ^{125}I-protein A, a single band of 230 kDa was observed in the

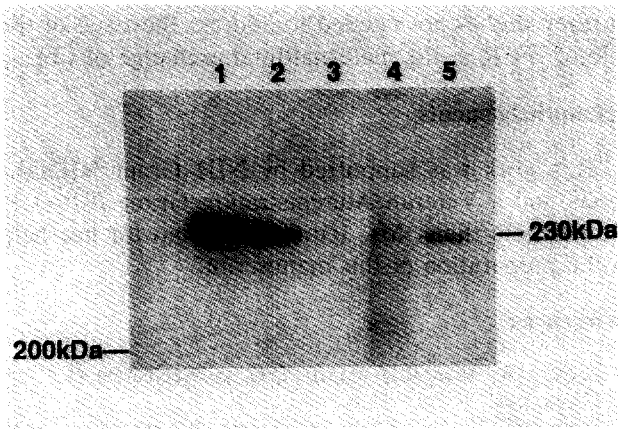

Fig. 3. Immunoblot of visceral yolk sac and placental membranes with rat anti-IFCR serum. Visceral yolk sac and placental membranes (100 μg protein) from 14-day-old fetus (lane 2), placenta (lane 4) and 19-day-old fetus (lane 3), placenta (lane 5) were separated on SDS-PAGE (5%). Lane 1, pure rat kidney IFCR. Further details of transfer and immunoblotting were carried out as described earlier [2].

14-day-old fetal and 19-day-old placental membranes (Fig. 3, lanes 2 and 5). The intensity of these bands coincided fairly well with the activity levels and had the same molecular mass as the pure rat kidney IFCR (Fig. 3, lane 1). A faint band was also observed with the 19-day-old visceral yolk sac membranes (Fig. 3, lane 4). However, no 230 kDa band was observed with 14-day-old placental membranes (Fig. 3, lane 3).

Cell free translation of visceral yolk sac mRNA

Immunoprecipitation of the cell free translation product (Fig. 4) of rat visceral yolk sac mRNA with anti-serum to pure kidney IFCR yielded on SDS-PAGE a single polypeptide of molecular mass 220 kDa (Fig. 4, lane 2). The identification of the band as IFCR was confirmed when anti-serum passed over a Sepharose-IFCR column failed to immunoprecipitate the same

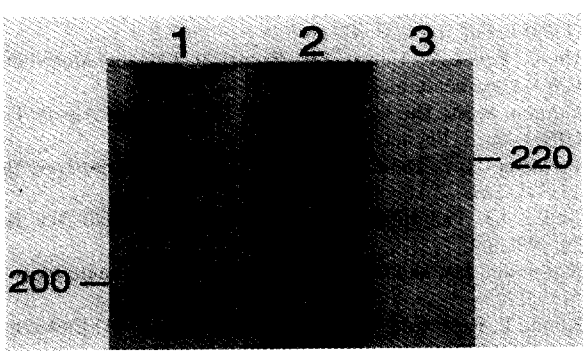

Fig. 4. Immunoprecipitation of [^{35}S]IFCR. [^{35}S]IFCR synthesized using poly(A$^+$)RNA (5 μg) from 14 (lane 2) and 19 (lane 1) day old pregnant rat yolk sac was translated in reticulocyte lysate system was extracted and immunoprecipitated with preabsorbed (lane 3) or normal anti-serum to rat kidney IFCR (lanes 1 and 2). The immune pellet was washed and subjected to SDS-PAGE (5%).

220 kDa protein (Fig. 4, lane 3). Similar translation using visceral yolk sac mRNA from 19-day-old pregnant rats did not yield an immunoprecipitatable IFCR band (Fig. 4, lane 1).

Discussion

The main objective of this study was to explore the possibility of IFCR expression in fetal derived membranes and to understand its possible role in the maternal-fetal exchange of Cbl. The results of the present study have shown that specific binding of IF-[^{57}Co]Cbl occurs to visceral yolk sac and placental membranes and this binding is due to expression of IFCR. Three evidences support this conclusion. First, the binding of IF-[^{57}Co]Cbl to visceral yolk sac membranes was inhibited by anti-serum raised to adult rat kidney IFCR (Fig. 1), but not to rat liver asialoglycoprotein receptor. Previous studies have shown that [^{57}Co]Cbl bound to another Cbl binding protein, haptocorrin, binds to asialoglycoprotein receptor which is expressed in the adult rat liver [17] and suckling rat intestine [18]. Furthermore, none of the sugars that inhibit the binding of mannose terminated glycoproteins to plasma membranes had any effect on the binding of rat IF-[^{57}Co]Cbl to the visceral yolk sac membranes. Although rat IF contains four high mannose type of N-linked oligosaccharides (Seetharam, B., unpublished observations) the lack of inhibition of IF-[^{57}Co]Cbl binding by mannose, mannan and *N*-acetylglucosamine suggest strongly that IF-[^{57}Co]Cbl binding to visceral yolk sac membranes is specific to IFCR, and not due to the binding to mannose/*N*-acetylglucosamine receptor. Moreover, recent studies [19] using recombinant rat IF have shown that absence of carbohydrate on IF has no effect on its binding to the receptor. Second, immunoblotting (Fig. 3) using fetal derived membranes identified on SDS-PAGE, a single band of 230 kDa in these membranes, while immunoprecipitation of in vitro translated IFCR reveals a molecular mass $\approx$ 220 kDa. These values are consistent with the core glycosylation of IFCR with 4 to 5 asparagine-linked oligosaccharides and their eventual processing to complex type during the maturation of rat IFCR [4,16]. Finally, the SDS-PAGE analysis of visceral yolk sac membranes did reveal a protein of 230 kDa, that co-migrated with pure rat renal IFCR (Fig. 2).

Another interesting aspect of this study, is the noted (Fig. 1) rise and fall in the specific activity of IF-[^{57}Co]Cbl binding to placental and visceral yolk sac membranes during the early and late gestation periods. It is interesting to note that earlier studies [20,21] have shown, not only fetal uptake of [^{57}Co] Cbl when bound to IF but also that this uptake dramatically declined ($>$ 90%) from 14-day to 19-day gestation periods. The decline in the in vitro fetal uptake of [^{57}Co] Cbl noted

in earlier studies agree with the decline in IFCR activity noted in the current study for the same periods of gestation. In addition, the increased IFCR activity in placental membranes from the 14th to the 19th day of pregnancy noted in this study, correlates well with the increased placental content of Cbl noted earlier [24] for the same period of gestation. These results suggest that IFCR may play a role in Cbl delivery to the fetus across these membranes throughout pregnancy. Although the factors which regulate IFCR expression are not known, placental lactogen and growth hormone have been shown to increase the IFCR activity in pregnant mother intestine during late gestation [22]. Whether a similar hormonal effect is responsible for the switching on or off, the IFCR expression in fetal membranes needs further study. Since the placental hormone levels do fluctuate throughout the gestation [23], it is possible that IFCR gene expression may be responsive to more than one hormone. The results (Fig. 4) suggest that IFCR gene expression may be regulated at the transcriptional level. Further studies are needed to validate this hypothesis.

Despite the current and previous observations on the regulation of IFCR activity in and uptake of [^{57}Co]Cbl from the fetal membranes, it is not known whether IF/IFCR uptake system for Cbl is active and important in vivo in the maternal-fetal exchange of Cbl during the various stages of pregnancy. This uncertainty is in part due to an incomplete understanding of the mechanisms of transplacental transport of Cbl during pregnancy. Earlier studies [24] have suggested that the maternal-fetal transport of Cbl via the placental barrier occurs in three stages. First, Cbl bound to plasma transporter, transcobalamin II (TC II) is transported across the maternal side of the placenta. Second, the transport of Cbl from the maternal to fetal side of the placenta, and third, transport of Cbl across the placental/fetal interface into the fetal plasma. In support of the first stage of transport receptor for TC II-Cbl has been identified [25] and isolated [26] from placental membranes. Following this initial uptake, how Cbl concentrated in the placenta crosses the fetal side of the placenta and visceral yolk sac membranes is not known. Based on earlier in vivo [24] and in vitro [20] studies in rats, the amount of Cbl transferred across these membranes, at all stages of pregnancy, do not exceed 1–1.5 ng/yolk sac or placenta per 60 min. Eventhough the turnover of IFCR is not known, the IF-Cbl binding capacity of 2–8 ng/yolk sac and 1 to 4 ng/placenta will be enough to transport 1–1.5 ng of Cbl/h. Whether IF is synthesized locally to help mediate Cbl transport across these membranes in rats is not known and needs further study.

In summary, the current studies have identified IFCR for the first time in the fetal derived membranes and show that its levels are regulated during gestation. Further studies are needed to address the issue of the role of IFCR in the maternal-fetal exchange of Cbl.

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