Brief Definitive Report

A Major Histocompatibility Complex Class I-like Fc Receptor Cloned from Human Placenta: Possible Role in Transfer of Immunoglobulin G from Mother to Fetus

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Summary

The acquisition of maternal antibodies is critical for immunologic defense of the newborn. In humans, maternal IgG is actively transported across the placenta. The receptor responsible for this transport has not been identified definitively. We report the isolation from a placental cDNA library of clones encoding the α-chain of an immunoglobulin G (IgG)-Fc receptor (hFcRn) that resembles a class I major histocompatibility complex antigen. The DNA and predicted amino acid sequences are very similar to those of the neonatal rat and mouse intestinal Fc receptors, rFcRn and mFcRn. These receptors mediate transport of maternal IgG from milk to the bloodstream of the suckling rat or mouse. Like rat and mouse FcRn, hFcRn binds IgG preferentially at low pH, which may imply that IgG binds hFcRn in an acidic intracellular compartment during transport across the placenta.

Most materno-fetal IgG transport occurs after the 22nd week of human pregnancy (1). During this time there are two cellular barriers in the placenta that IgG must cross: the syncytiotrophoblast and the fetal capillary endothelium. The microvillar surface of the syncytiotrophoblast is in contact with maternal blood. In some regions of the placenta the syncytiotrophoblast is separated from the endothelium of the fetal vessels only by a basement membrane; elsewhere mesenchymal cells of the villous stroma and remaining cytotrophoblast intervene.

The identity of the receptors that carry IgG across the human placenta is not known. Several IgG-binding proteins have been isolated from placenta (2, 3). FcγRII has been detected in placental endothelium (4–7) and FcγRIII in syncytiotrophoblast (6, 7). Both of these receptors have relatively low affinities for monomeric IgG and function on leukocytes as receptors for immune complexes (8). Placental alkaline phosphatase has also been shown to bind IgG (9).

IgG has been observed in transport vesicles in neonatal rat intestinal epithelium (10). Rat FcRn, which mediates intestinal IgG transport, consists of two subunits: β2-microglobulin (β_2m) and a large subunit that resembles MHC class I α chains (11). Late in gestation, a similar or identical FcRn is present in the fetal yolk sac of rat (12) and mouse (13). IgG has also been detected by immunoelectron microscopy in vesicles in the human syncytiotrophoblast (14, 15) and endothelial cells (15). These vesicles are presumed to mediate transcytosis of IgG in placenta.

We report the isolation of cDNA encoding a human homolog of the rat and mouse IgG transporting Fc receptor, FcRn. The presence of hFcRn RNA in the human placenta strongly suggests that this receptor functions in IgG transport in humans.

Materials and Methods

Cloning. A human genomic DNA fragment containing a sequence similar to that encoding the α2 domain of rat FcRn was first cloned using a rFcRn cDNA probe (Mikulska, J. E. and N. E. Simister, unpublished results). A λgt11 cDNA library of human full term placenta (Clontech, Palo Alto, CA) was probed at high stringency (final wash 60°C, 0.1× SSC, 0.1% SDS) with a fragment of human genomic DNA encoding the α2 domain of a homolog of rat and mouse FcRn.

Sequence Determination and Analysis. DNA sequencing was done by thermal cycle sequencing using the Circumvent™ Kit (New England Biolabs, Beverly, MA). Contig assembly was done using a prerelease version of SeqApp by Don Gilbert (Indiana University, Bloomington, IN). Multiple sequence alignment was done on a DEC VAX using the GCG package (16).

Expression and Binding Assay. hFcRn clone #3 was subcloned into the pREP9 eukaryotic expression vector (Neo^r; Invitrogen, San Diego, CA). 5 μg DNA was transfected into one 10 cm plate of 293 cells using a $CaPO_4$ method (17). Cells were diluted and placed under G418 selection. Individual G418-resistant colonies were expanded for binding assays. The presence of hFcRn transcripts in these lines was confirmed by Northern blots (data not shown).

Fc and IgG were purchased from Jackson Immunoresearch (West

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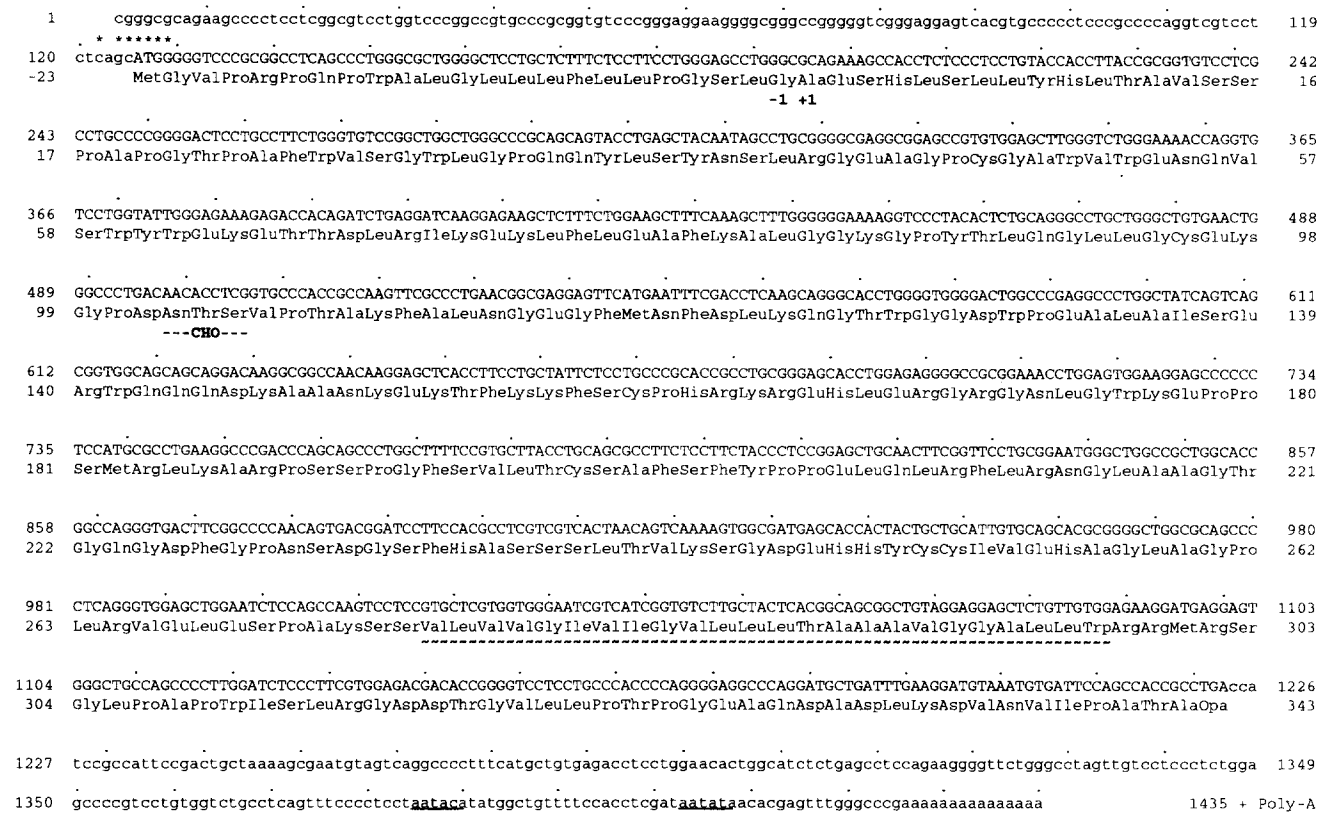

Figure 1. The complete nucleotide and deduced amino acid sequence of hFcRn α chain. The potential ATG start is marked over the initiation site consensus nucleotides (27). The most probable NH_2-terminus after signal peptide cleavage (28) is indicated by +1. A potential site of Asn-linked glycosylation (Asn-X-Ser), the predicted hydrophobic membrane-spanning segment and polyadenylation signals are underlined. These polyadenylation signals have each been reported to be ~10% as efficient as the canonical AATAAA signal in polyadenylation assays *in vitro* (29). These sequence data are available from EMBL/GenBank/DDBJ under accession number U12255.

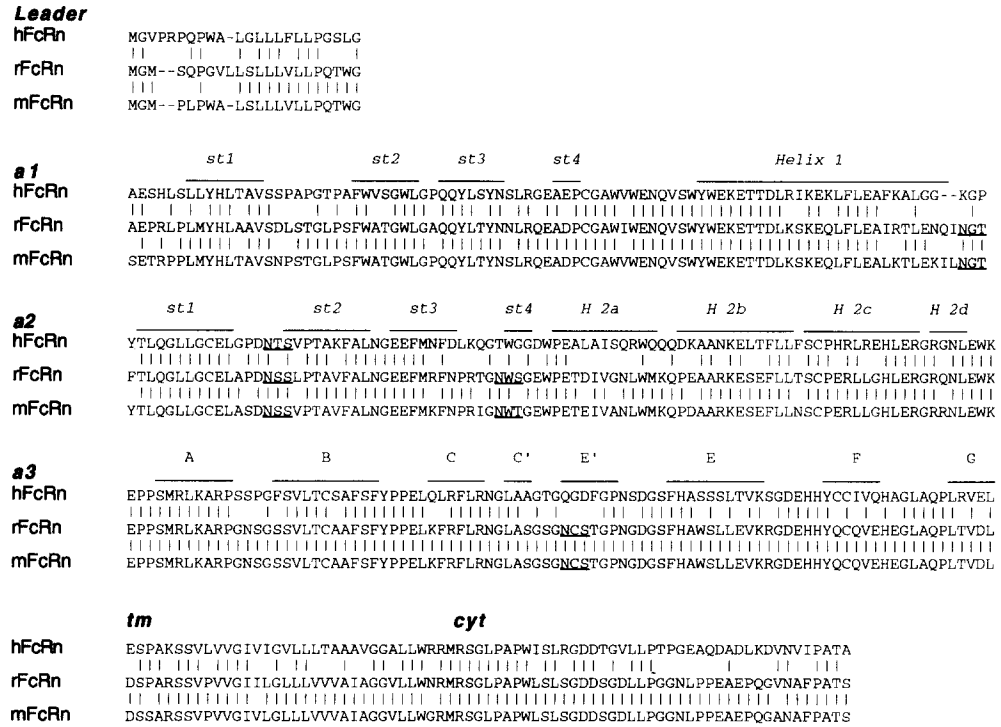

Figure 2. Domain by domain comparison of the predicted amino acid sequence of hFcRn with rFcRn and mFcRn. Identities are indicated by a vertical bar. Labeled bars over the sequence indicate ordered secondary structure elements assigned based on the crystal structure of rFcRn (30). The sequence data for hFcRn are available from EMBL/GenBank/DDBJ under accession number U12255.

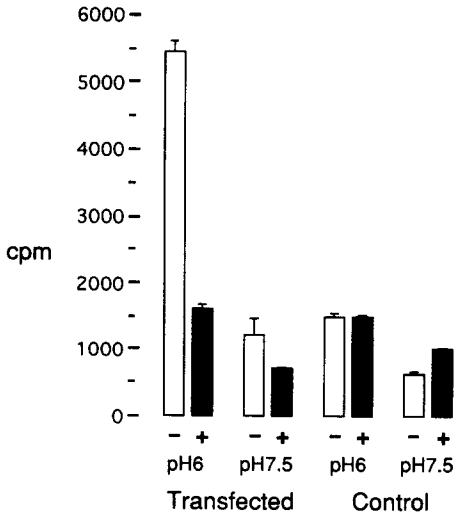

Figure 3. Fc binding by human FcRn. Binding of ^{125}I-Fc by 293 cells stably transfected with hFcRn α-chain cDNA in the expression vector pREP9 (Invitrogen) or untransfected. Assays were done at 37° with (*filled columns*) and without (*open columns*) competing unlabeled IgG, at pH 6.0 and 7.5. Each column represents the mean cell-associated radioactivity of three wells; bars indicate the SE.

Grove, PA). Fc fragment of human IgG was labeled with Na^{125}I to a specific activity of ~0.5 Ci/μmol using Iodogen® (Pierce, Rockford, IL).

Cells expressing the hFcRn and nontransfected controls were seeded into 12-well plates at 10^5 cells/well and assayed 2 d later when they had reached near confluence. The cells were first washed with DMEM, pH 6.0 or 7.5. Then, ^{125}I-Fc (200 ng/ml, 4×10^{-9} M) in DMEM, 1 mM KI, 1% fish gelatin (Sigma Chemical Co., St. Louis, MO), 20 mM Hepes, pH 6.0 or 7.5 with or without 0.5 mg/ml unlabeled human IgG (3.3×10^{-6} M) was added. The cells were allowed to bind and take up IgG/Fc for 2 h at 37°C. Cells were then cooled to 4°C, and unbound ligand was removed with three washes of chilled PBS, 1 mM KI, pH 6.0 or 7.5. Cells were dissolved in 0.1 N NaOH and transferred to vials. Bound radioligand was measured in a gamma counter (CliniGamma model 1272; LKB Wallac, Piscataway, NJ).

Northern Blots. A human multiple tissue Northern blot was purchased from Clontech. For a second blot, total cellular RNA from human tissues (10 μg/lane) was run on a denaturing agarose gel and transferred to a nylon membrane (Biotrans™; ICN, Irvine, CA). RNA was cross-linked to the membrane by UV illumination (Stratalinker®; Stratagene, La Jolla, CA). Both blots were hybridized with 2×10^6 cpm/ml of ^{32}P-labeled probe made by a random primer method (Prime-it® RmT, Stratagene) and passed through a 1-ml G25 spin column to remove free nucleotides. The blot was hybridized for 1 h in Quickhyb® (Stratagene) at 68°C; the final wash was 0.1× SSC, 0.1% SDS at 60°C. The blot was exposed on a phosphor screen (Kodak, Rochester, NY) and analyzed on a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Results and Discussion

A human genomic DNA fragment containing a sequence similar to that encoding the α2 domain of rat FcRn was first cloned using a rFcRn cDNA probe. We used this human DNA to probe a human placental cDNA library, and isolated five independent cDNA clones. Three of these contained an identical 1,098-nucleotide open reading frame (ORF, Fig. 1); two lacked the first 351 bases of the ORF. The ORF was followed by 212 bp of 3′-untranslated sequence including two potential polyadenylation signals: AATATA at −17 and AATACA at −47 nucleotides upstream of a poly(A) tail.

The human cDNA sequence and its deduced amino acid sequence were very similar to those of the rFcRn α chain, with 69% nucleotide identity over the ORF, and 65% predicted amino acid identity (Fig. 2). The amino acid identity between the rat and human homologs was 65% in the α1 domain, 64% in α2, 73% in α3, 63% in the transmembrane region, and 55% in the cytoplasmic tail. Only one of the four potential sites for N-linked glycosylation shared by rat and mouse FcRn was present in the human protein, at Asn 102. Notably absent was the site in α1, Asn 87 in rFcRn, that is almost ubiquitous in class I MHC proteins. The loop in which this site would lie was two residues shorter in human FcRn than in the rat and mouse receptors. One of the two aromatic residues in the cytoplasmic region of rFcRn and mFcRn was present in the human protein (Trp 309). A dileucine motif (positions 320 and 321) was also conserved. Both of these elements are potential signals for endocytosis (18).

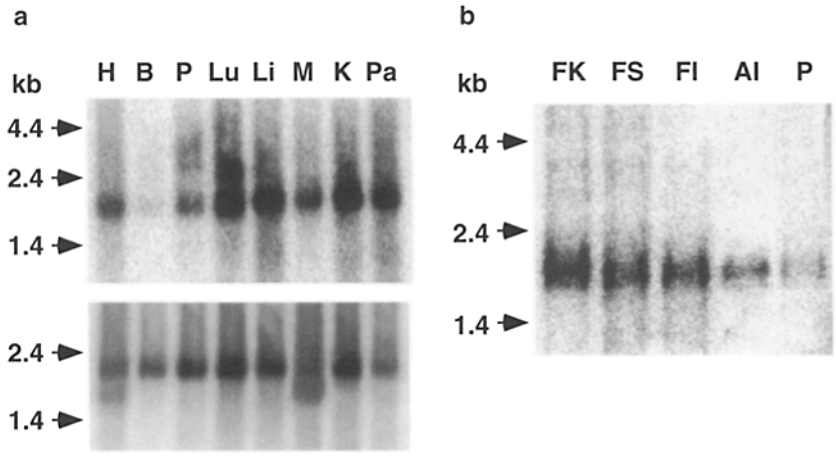

Figure 4. Northern blots of RNA from human tissues. (*a*) Human multiple tissue Northern blot (Clontech) hybridized with a 120-bp probe from the 3′ untranslated sequence of hFcRn (*top*) and with a β-actin control probe (*bottom*). Tissues are heart (*H*), brain (*B*), placenta (*P*), lung (*Lu*), liver (*Li*), skeletal muscle (*M*), kidney (*K*) and pancreas (*Pa*). (*b*) Northern blot of human RNA hybridized with a 120-bp probe from the 3′ untranslated sequence of hFcRn. Tissues are fetal kidney (*FK*), fetal spleen (*FS*), fetal intestine (*FI*), adult intestine (*AI*), and placenta (*P*). Loading of lanes was approximately equal, as judged by ethidium bromide staining of ribosomal RNAs (not shown).

To determine whether the human clones encoded an Fc receptor, we transfected one of the cDNAs into the human 293 cell line and measured the binding of radiolabeled human Fc. Cells that expressed hFcRn bound Fc specifically at pH 6.0 but not at pH 7.5; untransfected cells showed no specific binding at either pH (Fig. 3). The same pH dependence of binding has been observed for rFcRn (19). The optimum pH for IgG binding to placental membranes has also been reported to be between 5 and 6.5 (20, 21). The pHs of maternal and fetal blood are close to neutral (22) and that of stroma is expected to be similar, so hFcRn could not bind IgG at the surface of the syncytiotrophoblast or endothelium. A similar situation exists in the rat fetal yolk sac endoderm, where FcRn appears to transport IgG without a pH gradient (12). In this tissue, FcRn is detected not at the apical plasma membrane but in apical vesicles. It has been suggested that these are acidified endocytic vesicles where IgG that was taken up by fluid-phase endocytosis binds FcRn (12). A similar mechanism has been proposed for placental IgG transport (15). The ability of hFcRn to bind IgG at acidic but not neutral pH would enable this receptor to transport IgG by binding it in endosomes of syncytiotrophoblast or in the endothelium of placental vessels and releasing it into the stroma or fetal blood.

Finally, we examined the tissue distribution of hFcRn α chain mRNA using Northern blots. A 1.5-kb transcript was present in RNA from full-term human placenta, and also heart, lung, liver, muscle, kidney, pancreas, and both fetal and adult small intestine (Fig. 4). The signal did not represent crosshybridization with class I MHC mRNA because it was detected with a probe from the 3′ untranslated region of hFcRn which is unlike the class I sequences. The mRNA detected in fetal intestine is likely to encode the receptor responsible for the pH-dependent IgG binding activity in this tissue (23). Placental endothelial cells make β_2m (24). Syncytiotrophoblast lacks classical class I MHC proteins and was reported to contain no β_2m (24), but recent reevaluation suggests that β_2m is present, although nonuniformly distributed (J. S. Hunt, personal communication). Thus, functional FcRn could be assembled in endothelial cells or in β_2m-positive regions of syncytiotrophoblast.

The expression in human placenta of a homolog of the rodent IgG-transporting Fc receptor strongly implicates hFcRn in IgG transport across the placental barrier. Future identification of the placental cell types in which hFcRn is expressed, by in situ hybridization or immunohistochemistry, will be necessary to clarify its role. The clinical importance of maternal IgG for the immunologic defense of the neonate is well established. Not all antibodies are beneficial, however. Maternal IgG antibodies resulting from isoimmunization by fetal erythrocytes, leukocytes, or platelets (25) and autoantibodies in SLE and other autoimmune diseases of the mother (26) may harm the fetus. Identification of the Fc receptor that transports maternal IgG to the fetus would be a first step toward rational intervention in pregnancies complicated by these conditions.

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