Available online at www.sciencedirect.com
ScienceDirect
PLACENTA
Placenta 28 (2007) 170–174

Endothelial Expression of Fc Gamma Receptor IIb in the Full-term Human Placenta

T. Mishima [a], G. Kurasawa [a], G. Ishikawa [b], M. Mori [a], Y. Kawahigashi [a], T. Ishikawa [a], S.-S. Luo [a], T. Takizawa [a], T. Goto [a], S. Matsubara [c], T. Takeshita [b], J.M. Robinson [d], T. Takizawa [a,*]

[a] *Department of Molecular Anatomy, Nippon Medical School, 1-1-5 Sendagi, Tokyo 113-8602, Japan*
[b] *Department of Obstetrics and Gynecology, Nippon Medical School, 1-1-5 Sendagi, Tokyo 113-8602, Japan*
[c] *Department of Obstetrics and Gynecology, Jichi Medical School, Tochigi 329-0498, Japan*
[d] *Department of Physiology and Cell Biology, Ohio State University, Columbus, OH 43210, USA*

Accepted 31 January 2006

Abstract

In the third trimester, human placental endothelial cells express Fc gamma receptor IIb (FcγRIIb). This expression is unique because FcγRIIb is generally expressed on immune cells and is typically undetectable in adult endothelial cells. Recently, we found a novel FcγRIIb-defined, IgG-containing organelle in placental endothelial cells; this organelle may be a key structure for the transcytosis of IgG across the endothelial layer. In this study, we verify the expression of FcγRIIb in endothelial placenta cells and use reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing analyses to define the expressed *FCGR2B* mRNA transcript variant. We also investigated the distribution of *FCGR2B* mRNA and protein within the vascular tree of the full-term human placenta by RT-PCR and quantitative microscopy. The mRNA sequence of *FCGR2B* expressed specifically in placental endothelial cells is that of transcript variant 2. FcγRIIb expression and synthesis occur throughout the placental vascular tree but do not extend into the umbilical cord. This study provides additional information on FcγRIIb expression in the human placenta.

Keywords: Human; Fc receptors; Reproductive immunology; Endothelium; Placenta

1. Introduction

Human Fc gamma receptor IIb (FcγRIIb) is a single-chain, low-affinity IgG receptor that is widely expressed on immune cells such as B cells and macrophages (reviewed in Ref. [1]). The FcγRIIb negatively regulates multiple cell signaling pathways in the immune system via the immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (reviewed in Ref. [2]). Although FcγRIIb is encoded by a single *FCGR2B* gene, located on human chromosome 1q23, the two common isoforms, b1 and b2, are generated by alternative splicing of the corresponding mRNA sequences [3]. Three separate exons in the gene encode the cytoplasmic domain of FcγRIIb. The major difference between the mRNA sequences of the b2 and b1 isoforms is that b2 lacks the first cytoplasmic exon (C1 exon), whereas it is retained in the b1 isoform.

The distribution of the various Fc receptors has been studied in the human placenta (reviewed in Ref. [4]). It has been reported more recently that human placental endothelial cells express FcγRIIb in the third trimester [5]. Although the details of the specific function(s) of FcγRIIb and its regulatory role in pregnancy are still unresolved, we have recently shown that FcγRIIb is expressed in a novel IgG-containing organelle.

* Corresponding author. Tel.: +81 3 3822 2131; fax: +81 3 5685 3052.
E-mail address: t-takizawa@nms.ac.jp (T. Takizawa).

0143-4004/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2006.01.024

We hypothesize that this is a key intracellular structure for the transcytosis of IgG across placental endothelial cells to reach the fetal circulation [6]. In these cells, most of the intracellular IgG (~80%) is associated with the FcγRIIb-defined organelle. This newly described compartment is the most abundant membrane-bounded organelle in the placental endothelial cells, and it is distinct from the morphologically similar caveolae. The expression of FcγRIIb in placental endothelial cells is unusual in two regards. First, FcγRIIb is not typically present in endothelial cells [7]. Additionally, we demonstrated that FcγRIIb expression was restricted to an intracellular organelle within placental endothelial cells, whereas FcγRIIb primarily has a cell surface distribution in immune cells [6]. Based on immunoblot analyses, Lyden et al. proposed that the b2 isoform of FcγRIIb is expressed in placental endothelial cells [5]. In the present study, we use reverse transcriptase-polymerase chain reaction (RT-PCR) and sequence analyses to determine the transcript variant of *FCGR2B* mRNA expressed in placental endothelial cells. We also investigated the expression of *FCGR2B* mRNA and protein within the vascular tree of the full-term human placenta by RT-PCR and quantitative immunofluorescence microscopy. This study provides additional insight into the unique expression of FcγRIIb and its potential role in the human placenta.

2. Materials and methods

2.1. Sample collection

Human full-term placentas were obtained according to a protocol approved by the Nippon Medical School Hospital Ethics Committee. Tissue samples from uncomplicated Cesarean deliveries were processed as soon as possible following delivery (within 20 min). Three placentas were used for *FCGR2B* transcript variant analysis and three other placentas for the quantitative immunofluorescence microscopy analysis.

2.2. Cloning of FCGR2B transcript variants 1 and 2

White blood cells were used to obtain clones of *FCGR2B* transcript variants 1 (*FCGR2B1*) and 2 (*FCGR2B2*), as FcγRIIb-specific mRNAs are abundant in these cells [8]. Whole human blood was collected from healthy male volunteers after obtaining informed consent, and neutrophil and monocyte/lymphocyte fractions were isolated from the whole blood samples as described previously [9]. Total RNA was extracted from the isolated cells using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The cDNA was synthesized using 500 ng of total RNA, MultiScribe reverse transcriptase (Applied Biosystems, Foster City, CA), and an oligo(dT) primer (Invitrogen, Carlsbad, CA). DNA amplification was performed by PCR using ExTaq DNA polymerase (TaKaRa BIO, Shiga, Japan) and the following FcγRIIb-specific primers: 5′-GGAATTCCATGGGAATCCTGTCATTCT TACCTGTC-3′ (forward) and 5′-GGGGTACCCCCTAAATACGGTTCTGGT CATCAGGC-3′ (reverse). These primers allow the amplification of full-length FcγRIIb and include restriction sites for *Eco*RI (forward) and *Kpn*I (reverse). The PCR conditions were as follows: initial denaturation for 2 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C, with a final extension step of 5 min at 72 °C. The PCR products were digested with *Eco*RI and *Kpn*I (TaKaRa BIO) at 37 °C for 2 h, and the fragments were separated by electrophoresis on a 2% agarose gel stained with SYBR Green I (Molecular Probes, Eugene, OR). The bands containing *FCGR2B1* (951 bp) and *FCGR2B2* (894 bp) were removed from the agarose gels, and the DNA purified using a Gene Clean III kit (Q-BIO Gene, Carlsbad, CA). These PCR products were inserted into the EGFP-C1 plasmid vector (BD Biosciences Clontech, Mountain View, CA) and cloned. To confirm that the vectors included the full coding regions of *FCGR2B1* and *FCGR2B2*, the inserts of the cloned plasmids were sequenced using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction mix (Applied Biosystems) according to the manufacturer's instructions, with an ABI 310 sequencer (Applied Biosystems). The sequences were assembled and compared using Vector NTI v5 software (InforMax, North Bethesda, MD). The plasmids containing *FCGR2B1* and *FCGR2B2* were used as positive control templates for RT-PCR analysis.

2.3. RT-PCR analysis of FCGR2B mRNA variants in human placental endothelial cells

To determine the *FCGR2B* transcript variant expressed in fetal endothelial cells, terminal villus (TV)-rich pellets were used. A modification in the method of Kacemi et al. [10] was used to collect the TVs. Briefly, following extensive washing with cold PBS (0.15 M PBS, pH 7.4), fresh 1- to 2-cm^3 blocks of placental tissue were placed in cold Hank's balance salt solution (HBSS) and were progressively diced by cross-cutting with paired disposable microtome blades; this released large numbers of very small villus pieces into suspension. After dissection, large pieces containing stem villi were strained from the suspended fragments using a 30-mesh stainless-steel soup strainer (Endo Shoji, Tsubame, Japan). Blood cells in the strained suspension were removed using a 30-μm diameter nylon filter net (Nippon Rikagaku Kikai, Tokyo, Japan) that allows blood cells to pass through but retains the TVs. The TVs were collected by resuspending in HBSS followed by centrifugation (5 min, 230 × *g*, 4 °C). After three washes in PBS, the TV-rich fraction was prepared by centrifugation (30 s, 8000 × *g*). The morphology of the sample contents was determined by differential interference contrast (DIC) microscopic examination.

For the comparative analysis of *FCGR2B* mRNA expression in different locations of the placental vascular tree, portions (2–3 cm in length) of fetal vessels from the chorionic plate (chorionic vessels) and from the umbilical cord were dissected out and cut open lengthwise. Endothelial sheets were stripped off of the vessels under a stereomicroscope using style 5 tweezers (Ted Pella, Redding, CA).

Total RNA extraction, cDNA synthesis, and PCR conditions for the RT-PCR analyses were performed as described above. The primer sequences for *FCGR2B* (NCBI reference: NM_001002273) and *GAPDH* (NM_002046) were: *FCGR2B* (196 bp, *FCGR2B1*; 137 bp, *FCGR2B2* [8]), 5′-CCAATGGGG ATCATTGTGGC-3′ and 5′-CCCAACTTTGTCAGCCTCATC-3′; and *GAPDH* (373-bp PCR product), 5′-GGTCGTATTGGGCGCCTGGTCACC-3′ and 5′-CACACCCATGACGAACATGGGGGC-3′.

To confirm the *FCGR2B* mRNA variants expressed in placental endothelial cells, we also performed direct sequencing analysis. The full coding region of *FCGR2B* was amplified by PCR using the TV cDNA and the *FCGR2B* cloning primers (see Section 2.2). The PCR product was purified on an agarose gel and confirmed by sequence analysis.

2.4. Quantitative immunofluorescence microscopy

For immunofluorescence microscopy, small blocks of placental tissue (approximately 1 cm^3) were harvested, cut into thin slices, and fixed in 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, containing 5% sucrose for 2 h at 22 °C. Umbilical cords (approximately 2–3 cm from the placental insertion) were also dissected out and processed in the same manner as described above.

Tissue sections (5-μm thick) were cut with a Microm HM 550 cryostat (Microm, Walldorf, Germany), mounted on round glass cover slips (13-mm diameter, No. 1 thickness; Matsunami, Osaka, Japan), coated with 2% 3-aminopropyltriethoxy-silane (Sigma Chemical Co., St. Louis, MO), and allowed to air dry. The sections were washed three times in PBS and then incubated in 1% bovine serum albumin and 5% normal goat serum in PBS for 1 h at room temperature, to block non-specific protein-binding sites. For double labeling, the tissue sections were incubated with the primary antibodies, rabbit anti-FcγRIIb (Ab 163.96, 1:3,200 dilution [6]) and mouse anti-CD31 (PECAM 1.3, 6.9 μg/ml [11] or clone 9G11, 10 μg/ml, R&D, Minneapolis,