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Dynamics of immunoglobulins at the feto–maternal interface

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Transplacental transport of maternal immunoglobulin G (IgG) to the developing fetus is extremely important in the protection of the newborn from infection. Although the exact mechanisms of the selective and active transfer of IgG across the placental barrier are not fully understood, receptors for the Fc part of IgG (FcγRs) in the placenta are believed to play a key role. Several known Fc receptors, FcγRI, FcγRII, FcγRIII and FcRn (neonatal FcR), demonstrate heterogeneous expression patterns in placenta. Immunohistochemical analysis shows the expression of FcγRI on Hofbauer cells in stromal tissue, FcγRII on Hofbauer cells and fetal blood endothelium, FcγRIII on Hofbauer cells and trophoblasts, and FcRn on syncytiotrophoblasts and endothelial cells. Recent studies provide evidence for important associations among these receptors and transcytosis of IgG, as well as scavenger mechanisms for clearing immune complexes in the placenta during pregnancy.

Humoral-mediated immunity is transferred passively from mother to fetus during mammalian pregnancy. In humans, substances that pass from maternal blood to fetal blood must traverse the histological barrier, which consists of the syncytiotrophoblast, the stroma of the intravillous space, and the fetal capillaries. Although this barrier separates the blood in the maternal and fetal circulation, it is not a simple physical barrier. A wide range of substances is transferred actively or passively through the placenta to the fetus. Most low molecular mass compounds (< 500 Da) simply diffuse through the placental tissue interposed between the maternal and fetal circulation. Some low molecular weight substances, such as ions and amino acids, show unidirectional transfer across the placenta. Substances of very high molecular weight do not usually traverse the placenta, but there are a few exceptions such as immunoglobulin G (IgG) with a molecular mass of about 160 kDa.

The immunoglobulins in the fetus consist almost totally of maternal IgG and are transferred across the placenta by means of a specific receptor-mediated mechanism. IgG transport from mother to fetus begins at about week 16 of gestation and increases thereafter. Before week 16 of gestation, serum IgG concentrations of the fetus are < 8% of the average normal adult concentration. Between weeks 17 and 22 of gestation, fetal serum IgG concentrations range between approximately 10 and 20% of the adult value, and, after week 22 of gestation increase rapidly, reaching maternal serum IgG concentrations by 26 weeks of gestation and maintaining them until delivery (Gitlin, 1971). The bulk of IgG is acquired by the fetus from the mother during the last 4 weeks of pregnancy, and fetal concentrations of IgG exceed maternal concentrations at full term. By the time of birth, fetal IgG1 concentration may exceed that of the mother. Fetal IgG2 concentration is often, but not always, lower than, and fetal IgG3 and IgG4 concentrations are equivalent to, maternal concentrations (Wang *et al.*, 1970; Hay *et al.*, 1971; Morell *et al.*, 1971, 1972; Virella *et al.*, 1972; Schur *et al.*, 1973; Chandra, 1976; Pitcher-Wilmott *et al.*,1980; Einhorn *et al.*, 1987; Malek *et al.*, 1994). Newborns begin to produce IgG slowly and adult values of IgG are normally attained by 3 years of age.

Immunoglobulin class-specific transfer of maternal antibodies to the fetus occurs because maternal IgG, but not IgM or IgA, crosses selectively into the fetal circulation. On the basis of the observation that whole IgG molecules or Fc fragments of IgG pass into the fetal circulation more readily than F(ab')2 fragments (Brambell *et al.*, 1960), it has been hypothesized that IgG Fc receptors (FcγRs) on placental cells may be involved in the mechanisms responsible for IgG specific transfer across the placenta. There are three known subtypes of FcγRs: FcγRI, FcγRII, and FcγRIII, on human leukocytes. Several isoforms of each subtype on myeloid cells have been also reported (for review, see Huizinga *et al.*, 1991; Ravetch *et al.*, 1991). This review focuses on the expression and localization of heterogeneous FcγRs, including leukocyte FcγRs and their isoforms, in human placental tissues, and possible mechanisms of FcγRs expressed on various cell components of placental villi for materno–fetal IgG transfer are discussed.

IgG-binding molecules in placenta

Various methods have been used to investigate IgG-binding molecules, such as FcγRs, in placenta. Most of them used the analysis of the binding of isolated human placental membrane extracts to radiolabelled human IgG or IgG-sensitized erythrocytes. Estimates of the affinity of monomeric IgG for receptors in placental membrane range from 4×10^6 to 4×10^8 mol l^{-1} and a total number of binding sites per mg placental membrane protein ranges from 2×10^{12} to 2×10^{14} (McNabb *et al.*, 1976; Brown and Johnson, 1981; Niezgodka *et al.*, 1981; Lubega,

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1990). The broad range of the association constants and the total number of receptor sites for IgG are due mainly to the heterogeneity of FcγRs expressed on placental cells, although the different methods used to prepare the membrane fractions may be partly involved.

Several investigators used affinity chromatography or precipitation of IgG complexes with membrane extracts from human placenta to isolate the placental FcγRs. Triton-X lysates of placental cell membrane that bind IgG conjugated Sepharose had apparent molecular masses of 37, 45 and 60 kDa (Balfour and Jones, 1978). This heterogeneity was also demonstrated in IgG-binding molecules of placental membrane glycoprotein fraction extracted with lithium di-iodosalicylate. This fraction contained 26–29, 52–56 and 64–67 kDa proteins under reducing conditions that were precipitated with human IgG (Niedzgodka *et al.*, 1981; Mikulska *et al.*, 1987). A 40 kDa FcγR has been purified using a mouse monoclonal antibody, designated as B1D6, which reacts with the apical aspect of trophoblast and the endothelium of the fetal stem vessels (Matre *et al.*, 1984, 1989). These results reflect the molecular heterogeneity of FcγRs in human placenta and indicate that heterogeneous expression of FcγRs may occur in the placenta, which is composed of a variety of cells, such as syncytiotrophoblasts, cytotrophoblasts, fetal endothelial cells and stromal cells, including immunocompetent cells (for review, see Saji *et al.*, 1994; Simister and Story, 1997).

The neonatal Fc receptor, designated as FcRn, has been identified in suckling rats (Rodewald and Kraehenbuhl, 1984; Simister and Rees, 1985; Simister and Mostov, 1989; Roberts *et al.*, 1990). This molecule, which mediates intestinal IgG transport, consists of two subunits: β_2-microglobulin and a large subunit that resembles MHC class Ia chains. A human homologue of rodent FcRn has been isolated from human full-term placenta, and its unique distribution in human placenta has been determined (Story *et al.*, 1994; Kristofferson and Matre, 1996; Leach *et al.*, 1996; Simister *et al.*, 1996).

General characteristics of human FcγRs

The receptors for the Fc domain of IgG, FcγRs, are some of the most important structures in the immune system, providing an essential link between the humoral response and the cell-mediated immune system. FcγRs interact with immunoglobulin, and a variety of biological responses, including phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity, release of inflammatory cytokines, and the enhancement of antigen presentation (Guyre *et al.*, 1989; Simms *et al.*, 1991). Monoclonal antibodies and cDNA probes have provided details of the structure of FcγRs. FcγRs are members of an immunoglobulin superfamily, and three main classes, FcγRI, FcγRII and FcγRIII, generating at least 12 different isoforms, have been recognized to date.

Three distinct but closely related FcγR classes have been mapped to chromosome 1 and some of their characteristics are summarized (Table 1; Fig. 1) (Anderson and Looney, 1986; Ravetch and Kinet, 1991; van de Winkel and Capel, 1993). FcγRI (CD64) is a 72 kDa glycoprotein with a high affinity for monomeric IgG that is constitutively expressed on monocytes–macrophages, and can be induced on neutrophils by interferon-γ stimulation (Perussia *et al.*, 1983). FcγRI possesses an extracellular region with three set immunoglobulin-like domains, a transmembrane region, and a cytoplasmic tail. A major difference in structure among FcγRI, FcγRII and FcγRIII is the third extracellular domain, which is found in FcγRI but not in FcγRII or FcγRIII. This unique structure may bind to monomeric IgG with a high association constant (Allen and Seed, 1989). Three highly homologous genes, FcγRIA, IB and IC, have been identified and mapped to the long arm of chromosome 1, band q21.1 (de Wit *et al.*, 1993). An important difference among the FcγRIA, IB and IC genes is located in the exon of the third extracellular domain, where stop codons are found in the FcγRIB and IC genes (van de Winkel *et al.*, 1991; Ernst *et al.*, 1992). FcγRIA generates a single transcript encoding a molecule, FcγRIa, while FcγRIB yields two transcripts, a full-length transcript (FcγRIb1), and one that lacks exon EC3 (FcγRIb2).

FcγRII (CD32) is a low-affinity receptor with a molecular mass of 40 kDa and is widely distributed on neutrophils, eosinophils, platelets, B cells and monocyte-derived cells. It is frequently expressed as the sole form of FcγR on cells. Complementary DNA analysis has revealed the existence of multiple isoforms with similar extracellular domains, a transmembrane region, and cytoplasmic domains of variable length. Six isoforms, FcγRIIa1, FcγRIIa2, FcγRIIb1, FcγRIIb2, FcγRIIb3 and FcγRIIc, have been identified (Stuart *et al.*, 1987; Hibbs *et al.*, 1988; Stengelin *et al.*, 1988; Clark *et al.*, 1989; Seki, 1989), encoded by a total of three genes, FcγRIIA, IIB and IIC, which are located on 1q23-24 (Brooks *et al.*, 1989; Grundy *et al.*, 1989; Cassel *et al.*, 1993). The products of FcγRIIA and IIB genes were found to differ in their signal peptides and cytoplasmic tails. Analysis of the gene structure showed the FcγRIIB and IIC genes to be highly homologous at their 5′ ends, while FcγRIIA and IIC were very similar in the 3′ regions. FcγRIIa, FcγRIIb and FcγRIIc are highly homologous in their extracellular domains, although FcγRIIa has two *N*-linked glycosylation sites, while FcγRIIb, and FcγRIIc have three. FcγRIIb differs considerably from FcγRIIa and FcγRIIc in the intracytoplasmic domain, sharing only the first eight amino acids.

FcγRIII (CD16) has a molecular mass between 50 and 80 kDa owing to extensive glycosylation. This low-affinity Fc receptor is expressed on neutrophils, eosinophils, natural killer (NK) cells, tissue macrophages and some T lymphocytes (Fleit *et al.*, 1982; Kimberley *et al.*, 1989). Two genes, FcγRIIIA and FcγRIIIB, have been identified and localized on 1q23-24, approximately 200 kb apart from the FcγRII gene complex (Simmons and Seed, 1988; Peltz *et al.*, 1989; Qiu *et al.*, 1990). The products of both genes encode proteins with extracellular regions consisting of two Ig-like domains. The FcγRIIIA gene encodes a transmembrane region with a 25 amino acid tail expressed in macrophages, NK cells, and some T cells. However, the FcγRIIIB gene product is linked to the outer leaflet of the plasma membrane via a phosphatidylinositol (PI)–glycan anchor, which is selectively expressed on polymorphonuclear cells (PMNs) (Ravetch and Perussia, 1989). The most obvious differences between FcγRIIIA and IIIB gene products are located at amino acid position 203 (Phe: FcγRIIIa; Ser: FcγRIIIb) and 204 (Arg: FcγRIIIa; stop codon: FcγRIIIb). A serine in FcγRIIIb determines a PI–glycan anchoring, whereas a phenylalanine preserves transmembrane and cytoplasmic regions and prevents PI–glycan linkage (Hibbs *et al.*, 1989;

Table 1. Characteristics of FcγR family

FcγR class (CD)	Molecular mass (kDa)	Affinity (mol l^{-1})	Distribution	Genes (chromosome)	Isoforms	mAbs
FcγRI (CD64)	72	10^8–10^9 (high)	Monocyte Macrophage	FcγRIA FcγRIB FcγRIC (1q21–1)	FcγRIa FcγRIb1^s, b2 FcγRIc^s	32.2 197 22 44 62 10.1
FcγRII (CD32)	40	1×10^6 (low)	Monocyte Macrophage Neutrophil Eosinophil Platelet, B cell Endothelial cell	FcγRIA FcγRIIB FcγRIIC (1q23–24)	FcγRIIa1, a2^s FcγRIIb1, b2, b3 FcγRIIc	IV3 2E1 KuFc79 CIKM5 KB61 AT10 41H16
FcγRIII (CD16)	50–80	$< 10^7$ (medium) 5×10^5 (low)	Macrophage LGL/NK cell Neutrophil	FcγRIIIA FcγRIIIB (1q23–24)	FcγRIIIa FcγRIIIb	3G8 Leu11a/b/c B73.1 CLB Gran1 CLB Gran11 VEP 13 GRM 1 MG38 1D3
FcRn	46	2×10^7–1×10^8 (high)	Intestine Yolk sac	FcRn		YFC 120.5 Anti – pep 54–74 112–125 2G3

mAbs, monoclonal antibodies; s, soluble receptor; LGN, large granular lymphocyte; NK, natural killer cell.

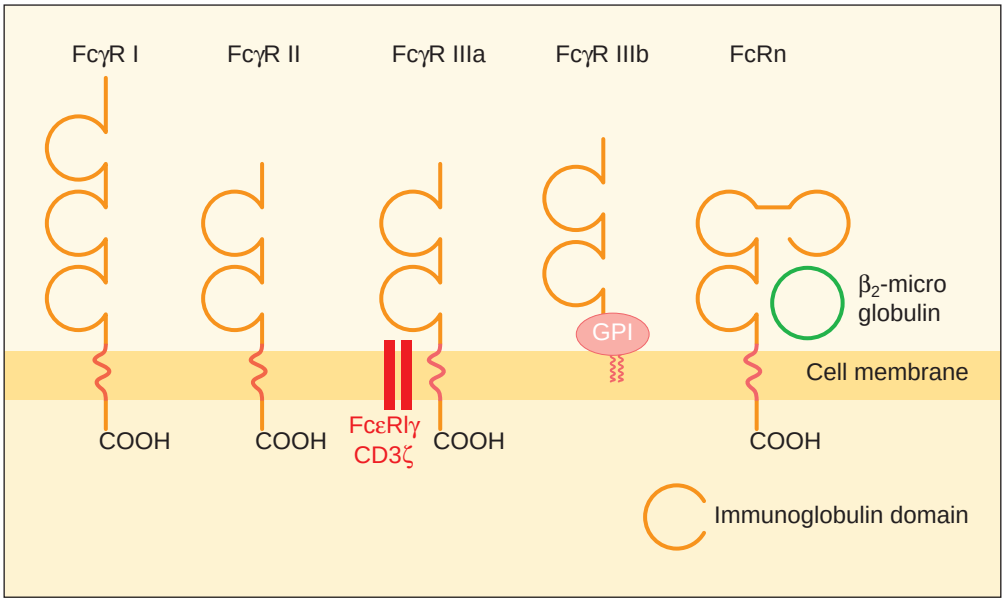

Fig. 1. Structure of human Fcγ receptors. GPI, glycosyl phosphatidylinositol.

Table 2. Localization of FcγRs in human term placenta

FcγR class (CD)	Reference	mAb	Hofbauer cells	Endothelial cells	Trophoblast
FcγRI (CD64)	Kristoffersen *et al.*, 1990	32.2	+	–	–
	Kameda *et al.*, 1991	32.2	+	–	–
	Sedmak *et al.*, 1991	32.2	+	–	–
	Wainwright and Holmes, 1993	32.2	+	–	–
	Bright *et al.*, 1994	10.1	+	–	–
FcγRII (CD32)	Stuart *et al.*, 1989	IV3	+	–	+
	Kristoffersen *et al.*, 1990	IV3	+	+	–
		CIKM5	+	+	–
	Micklem *et al.*, 1990	IV3	+	+	–
		2E1	+	+	–
		CIKM3	+	+	–
		CIKM5	+	+	–
		41H16	+	+	–
		KB61	+	+	–
	Kameda *et al.*, 1991	IV3	+	–	–
		2E1	–	+	–
	Sedmak *et al.*, 1991	IV3	+	+	–
		2E1	+	+	–
		KV79	+	+	–
		CIKM5	+	+	–
		41H16	+	+	–
		KB61	–	+	–
	Wainwright and Holmes, 1993	IV3	+	+	–
FcγRIII (CD16)	Kristoffersen *et al.*, 1990	3G8	+	–	+
		Leu11b	+	+	+
	Thaler *et al.*, 1990	Leu11a	ND	ND	–
		Poly Ab	ND	ND	+
	Kameda *et al.*, 1991	3G8	–	–	+
		Leu11b	–	–	+
	Sedmak *et al.*, 1991	3G8	+	–	+
		GLB Gran 1	+	–	–
		B73.1	+	–	–
	Wainwright and Holmes, 1993	YFC120.5	+	–	–
		GRAM1	+	–	–
		GLB Gran1	+	–	–
		3G8	+	–	–
		Leu 11b	+	+	+
FcRn	Kristoffersen and Matre, 1996	2G3	–	+/–	+
	Leach *et al.*, 1996	Anti – pep 54–74	–	+	+
	Simister *et al.*, 1996	Anti – pep 112–125	–	–	+

mAb, monoclonal antibody ; poly Ab, polyclonal anti–FcγRIII ; ND, not done.

Kurosaki and Ravetch, 1989; Lanier *et al.*, 1989). Expression of FcγRIII genes is highly regulated in a cell type-specific fashion, and the PI-linked FcγRIIIb isoform is expressed exclusively on PMNs. It is possible that FcγRIIIa transduces signals across the membrane independent of ligand-dependent engagement of FcγRIIa (Kimberley *et al.*, 1990; Edberg *et al.*, 1992; Hundt and Schmidt, 1992). The presence of FcγRIIIa in the membrane depends on its co-expression with the FcεRI γ chain or the ζ chain of the CD3–TCR (T-cell receptor) complex.

Localization of FcγR family in human placenta

FcγRI

As monoclonal antibodies raised against the myeloid FcγRs, FcγRI, FcγRII, FcγRIII, have become available, a number of immunohistochemical studies have been performed on the placenta. The results of these studies on human term placenta are summarized (Table 2; Fig. 2). In these studies, careful evaluation of control staining has been done, because the affinity between immunoglobulins and their specific antigens is usually

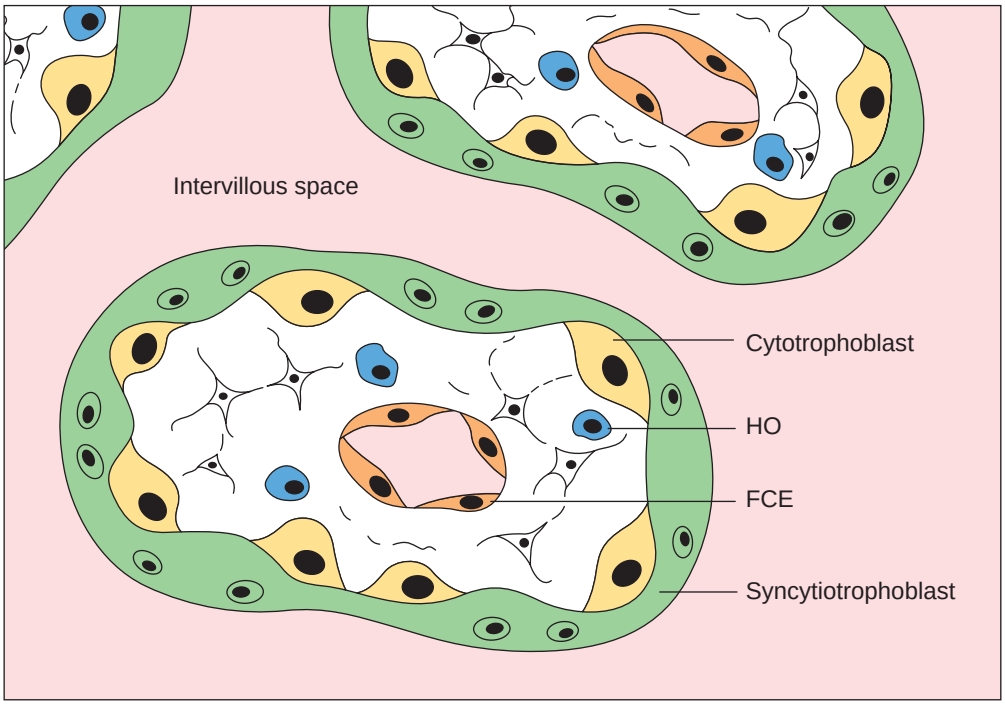

Fig. 2. Expression of Fcγ receptors on the chorionic villi. HO, Hofbauer cells; FCE, endothelial cells of fetal capillary. FcγRI (blue); FcγRII (dark orange); FcγRIII and FcRn (green).

10^4–10^7 mol l^{-1}, which is equivalent to the affinity of placental FcγRs for IgG. FcγRI has been found in the loose connective tissue and on Hofbauer cells but not on the trophoblast or endothelium of term placental villi (Kristoffersen *et al.*, 1990; Kameda *et al.*, 1991; Sedmak *et al.*, 1991; Wainright and Holmes, 1993; Bright *et al.*, 1994). All of the reports are in clear agreement on the positive expression of FcγRI on Hofbauer cells. Since Hofbauer cells are mononuclear phagocytes, morphologically defined as macrophages by phagocytosis and by IgG interaction, the expression of FcγRI on Hofbauer cells is consistent with its known expression on monocytes and macrophages.

FcγRII

Several isoforms of the FcγRII molecule have been identified and preferential recognition of one or more of numerous isoforms may underlie the different recognition patterns of FcγRII monoclonal antibodies. Kristofferson *et al.* (1990) and Micklem *et al.* (1990) showed that FcγRII is present on the Hofbauer cells of placental stroma and on the endothelial cells of fetal vessels in placental tissues by immunohistochemistry using six different monoclonal antibodies raised against FcγRII (IV3, 2E1, CIKM3, CIKM5, 41H16 and KB61). Sedmak *et al.* (1991) and Kameda *et al.* (1991) obtained similar results, although some inconsistent findings were observed. Kameda *et al.* (1991) demonstrated positive staining of Hofbauer cells with IV3 in the first and third trimester placenta, and 2E1 reactive endothelial cells in the third, but not the first, trimester placenta. Kristofferson *et al.* (1990), Micklem *et al.* (1990) and Sedmak *et al.* (1991) reported positive staining of placental macrophages and endothelium either by IV3 or 2E1 monoclonal antibody. The negative staining of endothelial cells by IV3 is consistent with the findings of Stuart *et al.* (1989). However, Stuart *et al.* (1989) revealed the positive IV3-staining of both Hofbauer cells and endothelium (Wainwright and Holmes, 1993). None of the antibodies discriminate absolutely between FcγRIIa, b and c isoforms. *In situ* hybridization methods, which may help to account for the inconsistent findings of the immunohistochemical analysis, have not been used for placental FcγRs.

In the study of the mRNA expression of three distinct classes of FcγR in human placenta, transcripts of the FcγRII gene showed unique expression patterns during pregnancy. FcγRIIB mRNA increased markedly in the placenta in the second and third trimesters, while FcγRIIA or FcγRIIC, like FcγRI and FcγRIII, were transcribed consistently throughout pregnancy (Koyama *et al.*, 1991). It is perhaps significant that the FcγRIIB gene transcript is detected only after week 20 of gestation, since the maternal IgG transfer to the fetus is rapidly increased after week 22 of gestation (Gitlin, 1971). Another interesting finding is the expression of FcγRII in the syncytiotrophoblasts of hydatidiform moles at mRNA and protein concentrations (Stuart *et al.* 1989), since FcγRII is not detected on the trophoblast cells of the normal placenta. This exceptional expression of FcγRII on trophoblasts may be a consequence of tumourous transformation.

FcγRIII

Several monoclonal anti-FcγRIII antibodies have been subjected to immunohistochemical analysis of its localization in human term placenta, and there are some conflicting results (Kristoffersen *et al.*, 1990; Thaler *et al.*, 1990; Sedmak *et al.*, 1991;

Kameda *et al.*, 1991; Wainwright and Holmes, 1993). There are conflicting reports for 3G8 and Leu11b antibodies. Various studies on other monoclonal antibodies, GLB, Gran1, B73.1, YFC120.5 and GRAM1, gave similar results showing reactivity to Hofbauer cells but not to trophoblasts or endothelial cells in term placenta. Kristoffersen *et al.* (1990) reported that the anti-neutrophil FcγRIII antibody, 3G8, showed a strong positive reaction with trophoblasts and a weak positive reaction with stromal cells, and that Leu11b antibody showed a positive reaction with stromal cells, endothelial cells and trophoblasts. Sedmak *et al.* (1991) reported a strong positive reaction of 3G8 with trophoblasts and inflammatory cells. However, Kameda *et al.* (1991) reported that both 3G8 and Leu11b antibodies stained trophoblasts in the first and third trimester placenta, but no staining of macrophages and endothelial cells was observed. Wainwright and Holmes (1993) demonstrated positive staining of 3G8 to Hofbauer cells but no staining of trophoblasts and endothelial cells. Leu11b reacted with trophoblasts as well as Hofbauer cells and fetal vessel endothelium.

One explanation for the different staining patterns of 3G8 is the contamination of another antibody, probably against alkaline phosphatase. It should be noted that the early batches of 3G8 may have been contaminated with anti-placental alkaline phosphatase. Placental alkaline phosphatase is a glycophosphatidylinositol (GPI)-linked protein present in villous and extravillous trophoblast and in the amniochorion in term placenta (Sakiyama *et al.*, 1979; Johnson and Molloy, 1983). Leu11b reacted consistently with trophoblasts either in the first trimester placenta and term placenta by immunohistochemical analysis. However, Leu11b does not appear to identify typical FcγRIII components in trophoblasts. On immunoblots of trophoblast membrane, Leu11b detects a 74 kDa protein band that is different from a typical FcγRIII band detected by the other antibodies, although all are in the same range reported for the heterogeneously glycosylated FcγRIII (Wainwright and Holmes, 1993). Nishikiori *et al.* (1993) prepared a trophoblast-enriched fraction from term placenta, reconstructed the membrane protein *in vitro* and then used the trophoblast-enriched fraction for flow cytometry. Indirect immunofluorescence of the purified placental trophoblast fraction showed positive staining of 3G8 and Leu11b but negative staining of anti-FcγRI antibody (32.2) or anti-FcγRII antibody (IV3), indicating that the FcγRIII determinant for leukocyte-derived monoclonal antibodies is, at least, present on term trophoblasts. In fact, cDNA encoding FcγRIIIa has been amplified by reverse transcriptase polymerase chain reaction (PCR) from RNA obtained from term trophoblasts (Nishikiori *et al.*, 1993).

Two similar genes, FcγRIIIA and FcγRIIIB, encode membrane glycoproteins that are anchored by phosphatidylinositol glycan and membrane-spanning polypeptides, respectively. FcγRIII cDNA from total RNA of placental trophoblasts was cloned by reverse transcriptase–PCR, and DNA sequences were determined to characterize the placental trophoblast-specific FcγRIII isoform (Nishikiori *et al.* 1993). The FcγRIII molecule on placental trophoblasts was encoded by the FcγRIIIA gene. These two distinct structures have been shown to have different susceptibilities against PI-specific phopholipase C (PLC), which can release proteins attached to the cell membrane by glycosyl–phosphatidylinositol anchors. In agreement with FcγRIII cDNA sequences derived from placental trophoblast mRNA, FcγRIII expressed on term placental trophoblasts is insensitive to PI-specific PLC. These findings are different from those reported for first trimester placental trophoblasts (Wainwright and Holmes, 1993). Treatment of first trimester trophoblast membranes with PI-specific PLC removes most of the anti-FcγRIII (GLB Gran1) reactive proteins, indicating that they represent PI glycan-linked FcγRIIIb. Leu11b reactive proteins are unaffected. Further investigation of this hypothesis is required.

FcRn

An MHC class I-like FcR has been cloned from human placenta (Story *et al.*, 1994). Similar FcR in the neonatal rodent gut, FcRn (neonatal Fc receptor), transports maternal IgG from ingested milk in the gut to the bloodstream of newborn mammals (Israel *et al.*, 1995). The intestinal FcRn from neonatal rats is a heterodimer of an integral membrane glycoprotein similar to MHC class I a-chain and β_2 microglobulin (β_2m) (Simister and Mostov, 1989). Mouse FcRn was detected in the neonatal small intestine and the fetal yolk sac, two tissues involved in IgG transport (Ahouse *et al.*, 1993).

The DNA and predicted amino acid sequences of human FcRn are very similar to those of the neonatal rat and mouse intestinal FcRn. Human FcRn α-chain shares 65% predicted amino acid sequences with the rat homologue (Story *et al.*, 1994). FcRn protein is expressed on syncytiotrophoblasts in human term placenta (Kristoffersen and Matre, 1996; Leach *et al.*, 1996; Simister *et al.*, 1996). FcRn is occasionally or weakly expressed on fetal vessel endothelium in placental tissues (Kristoffersen and Matre, 1996; Leach *et al.*, 1996) but it is not expressed on Hofbauer cells in the placenta. Immunohistochemistry showed FcRn staining in a granular fashion throughout the cytoplasm of the syncytiotrophoblasts but no staining of the apical trophoblast membrane was observed (Kristoffersen and Matre, 1996). When flow cytometry was used, the monoclonal antibody against FcRn did not stain the extracellular surface of the syncytiotrophoblast microvillous plasma membrane.

Like rodent FcRn, human FcRn binds IgG preferentially at low pH. The human FcRn expressing cell that transfected human FcRn cDNA bound the Fc fragment of IgG specifically at pH 6.0 but not at pH 7.5. The optimum binding of FcRn to IgG at low pH is reasonable for maternal milk IgG transport across the intestinal epithelium of rodents by FcRn. The pH in the duodenum and jejunum of neonatal rats is 6.0–6.5 (Rodewald, 1976). However, human FcRn could not bind IgG at the surface of the syncytiotrophoblast or endothelium, because the pH of maternal and fetal blood is close to neutral and that of stroma is expected to be similar. The role of FcRn in humans requires further study.

IgG transfer and Fc receptors in placental tissues

The transfer of maternal IgG through the human placenta must cross the syncytiotrophoblasts, the stroma of the intravillous space and the fetal vessel endothelium. These tissues express unique patterns of various types of Fc receptors of IgG including FcγR isoforms and FcRn. Trophoblast cells in term placenta express both FcγRIII and FcRn. Placental FcγRIII is a membrane-spanning FcγRIIIa isoform (NK cell type). The

binding of immune complexes, IgG binding on target cells, or anti-CD16 monoclonal antibody cross-linkage to membrane-spanning CD16 on NK cells induces rapid increases in intracellular Ca^{2+} and the hydrolysis of membrane phosphoinositides, resulting in the production of both inositol-1,4,5-triphosphate (IP_3) and IP_4 (Windebank *et al.*, 1988; Leibson *et al.*, 1990; Ting *et al.*, 1992). NK cell activation, mediated by FcγRIII cross-linkage, results in transcriptional activation of cytokines such as IFN-γ and TNF-α (Anegon *et al.*, 1988; Cassatella *et al.*, 1989). These observations indicate that the FcγRIIIa molecule on trophoblasts may bind immune complexes or antibody-coated particles in the maternal circulation and may associate with the transcription of lymphokines or the triggering of cell-mediated immunity.

FcRn on trophoblasts may be involved in the transport of IgG across the materno–fetal barrier in humans (for review, see Simister and Story, 1997). The hypothesis that FcRn is localized on syncytiotrophoblast is not supported by the immunohistochemical studies in which β_2m, one of the two polypeptide chains of which FcRn comprises, was not detected in syncytiotrophoblast (Faulk and Temple, 1976). In addition, although the finding that the optimum pH for the binding of FcRn to IgG is consistent with the previous observation that IgG binds optimally to placental membrane at pH 5.0–6.5 (Balfour and Jones, 1977; Watabe *et al.*, 1980), the intervillous spaces are not acidic but neutral. Furthermore, FcRn is not detected on syncytiotrophoblast apical plasma membrane (Kristoffersen and Matre, 1996). A similar situation occurs in the rat fetal yolk sac, where FcRn appears to transport IgG without a pH gradient. In this tissue, it has been suggested that acidified endocytic vesicles transport IgG (Roberts *et al.*, 1990). A similar mechanism has been proposed for placental IgG transfer. IgG binds in endosomes to the receptor that transports it across the syncytiotrophoblast (Leach *et al.*, 1991). Further investigation, using functional assays in trophoblast model systems, into the role of FcRn in the transplacental transport of IgG is required.

Fetal endothelial cells in placenta express FcγRII and FcRn, although data on FcRn expression on endothelium is still conflicting. The receptor-mediated process of transcytosis was confirmed by Sooranna and Contractor (1991) using cultured cytotrophoblast cells grown on filters that allow access to the apical and basal surfaces of the cells. This experiment showed that IgG transport was specific and occurred primarily in the apical-to-basal direction. A similar apical-to-basal transport pattern of IgG has been demonstrated in epithelial cells transfected by isoforms of murine FcγRII cDNA (Hunziker and Mellman, 1989; Miettinen *et al.*, 1989). Murine FcγRIIB2 mediates efficient ligand internalization, delivery to lysosomes, and apical-to-basolateral transport of IgG. The cDNA of human FcγRIIB, which is more than 60% homologous to murine FcγRIIB2 in its cytoplasmic domain and 3′-untranslated region, was amplified by PCR and the transcription of the FcγRIIB gene increased markedly at week 20 of gestation (Koyama *et al.*, 1991). As maternal IgG transfer begins at mid-trimester, FcγRIIB in placenta is a candidate molecule for the unidirectional transfer of IgG in the placenta after mid-trimester. However, FcγRII has not been detected on trophoblast layers that are in contact with the maternal blood. Furthermore, there is no evidence of co-operation between FcγRII and FcγRIII or FcRn for intraplacental IgG transfer. Once again, further analysis is required.

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