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Human IgG Fc receptor (hFcRII; CD32) exists as multiple isoforms in macrophages, lymphocytes and IgG-transporting placental epithelium

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We previously isolated cDNA clones from a human monocyte library that encoded one member of a family of low-affinity surface receptors for the Fc domain of IgG (hFcRII-A). To investigate possible structural and functional heterogeneity among these receptors, we have now isolated two additional cDNAs (hFcRII-B and hFcRII-C) from a human placental library, placenta being a good source of FcR-bearing macrophages and epithelial cells. Three cDNAs encoded related but distinct transmembrane glycoproteins containing two immunoglobulin-like domains; however, transfected cells produced receptors that were indistinguishable on the basis of ligand binding or reactivity with anti-hFcRII monoclonal antibodies. The sequences of hFcRII-A and -B were most closely related and were identical except for several amino acid substitutions and one small internal deletion. While the ectodomain of hFcRII-C was identical to hFcRII-B, its cytoplasmic tail was unrelated but highly homologous to the corresponding domain of the receptor isoform (mFcRII-B2) found in murine macrophages. Thus, human FcRII may be derived from at least two alternatively spliced genes. Northern blots revealed little difference in the pattern of expression of hFcRII isoforms among various myeloid and lymphoid cells or cell lines. However, the blots—as well as *in situ* hybridization and immunohistochemistry—demonstrated that hFcRII-C (along with a second monocyte marker, the c-*fms* encoded CSF-1 receptor) was expressed in placental syncytiotrophoblasts. Since syncytiotrophoblasts comprise the IgG-transporting epithelium of the placental villus, these findings suggest that FcR found in the immune system and in certain epithelia may be structurally or functionally related.

Key words: Fc receptor/IgG/isoforms/placental epithelium

Introduction

Cell surface receptors for the Fc portion of immunoglobulin play a central role in the vertebrate immune system by providing a bridge between the humoral and cellular aspects of the immune response. Members of the Fc receptor (FcR) family are specific for different classes of immunoglobulin and are expressed by almost all cells of the immune system (macrophages, many lymphocytes, platelets and granulocytes) as well as by certain transporting epithelia (e.g. placenta, liver and neonatal intestine) (Mellman *et al.*, 1988; Unkeless *et al.*, 1988; Kinet, 1989; Mellman, 1989). Accordingly, FcR exhibit a variety of cell type-specific functions including endocytosis, triggering the release cytotoxic and inflammatory mediators, the regulation of B-cell development, and the transcytosis of immunoglobulin across epithelial layers (Mellman *et al.*, 1988). Particularly in the case of receptors for IgG, recent work has demonstrated that this functional heterogeneity is partly due to the existence of considerable structural heterogeneity within a related family of receptors.

On human hematopoietic cells, three distinct classes of IgG FcRs (designated hFcRI, hFcRII and hFcRIII) can be distinguished on the basis of their different structures and ligand binding activities (Anderson and Looney, 1986; Unkeless *et al.*, 1988; Kinet, 1989; Mellman, 1989). All three classes are clearly members of the immunoglobulin gene superfamily. hFcRI, the only receptor that binds monomeric IgG with high affinity, is expressed primarily by macrophages and contains three immunoglobulin-like domains. hFcRIII, on the other hand, is expressed by a variety of leukocytes but binds monomeric and even aggregated IgG with very low affinity. Its extracellular region contains two immunoglobulin-like domains and it exists alternatively as a phospholipid-anchored or transmembrane protein (Selvaraj *et al.*, 1988; Simmons and Seed, 1988; Ravetch and Perussia, 1989; Scallon *et al.*, 1989). The most abundant and widely distributed receptor is hFcRII, being found on virtually all FcR-positive cells in the immune system. Like hFcRIII, hFcRII binds multivalent or aggregated IgG although with greater affinity and contains only two immunoglobulin-like domains. It, however, appears to be a transmembrane protein with a single membrane-spanning segment (Stuart *et al.*, 1987; Hibbs *et al.*, 1988; Stengelin *et al.*, 1988).

hFcRII is also the only class of human FcR for which a clear murine homologue has been identified (Mellman, 1989). In the mouse, however, mFcRII exists as at least three isoforms (mFcRII-A, -B1 and -B2) (Lewis *et al.*, 1986; Ravetch *et al.*, 1986; Hogarth *et al.*, 1987), each with its own characteristic cell type distribution and function. mFcRII-B1 and -B2, for example, are expressed predominantly in B-lymphocytes and monocyte-derived cells respectively (Ravetch *et al.*, 1986; T.Koch *et al.*, unpublished results). The two isoforms are identical except for an in-frame 47 amino acid insertion, presumably due to alternative mRNA splicing, in the cytoplasmic tail of mFcRII-B1. The cytoplasmic domain heterogeneity correlates with at least one important functional difference between the two receptors: only mFcRII-B2 is capable of localizing at clathrin-coated pits and rapid receptor-mediated endocytosis of bound ligand (Miettinen *et al.*, 1989). While the single human FcRII

hFcRII-B

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                                                    -42
                                                    MetGlyIleLeuSerPheLeuProValLeuAlaThrGluSerAspTrpAlaAspCysLysSerPro
                                  CTGTGCTCTGGGCGCCACTCGCTCCAGGGAGTGATGGGAATCCTGTCATTTTTACCTGTCCTTGCCACTGAGAGTGACTGGGCTGACTGCAAGTCCCCC
                          -15                                       -1 +1
GlnProTrpGlyHisMetLeuLeuTrpThrAlaValLeuPheLeuAlaProValAlaGlyThrProAlaAlaProProLysAlaValLeuLysLeuGluProGlnTrpIleAsnValLeu
CAGCCTTGGGGTCATATGCTTCTGTGGACAGCTGTGCTATTCCTGGCTCCTGTTGCTGGGACACCTGCAGCTCCCCCAAAGGCTGTGCTGAAACTCGAGCCCCAGTGGATCAACGTGCTC

 20                                                                                                                  50
GlnGluAspSerValThrLeuThrCysArgGlyThrHisSerProGluSerAspSerIleGlnTrpPheHisAsnGlyAsnLeuIleProThrHisThrGlnProSerTyrArgPheLys
CAGGAGGACTCTGTGACTCTGACATGCCGGGGGACTCACAGCCCTGAGAGCGACTCCATTCAGTGGTTCCACAATGGGAATCTCATTCCCACCCACACGCAGCCCAGCTACAGGTTCAAG

                                                                  80
AlaAsnAsnAsnAspSerGlyGluTyrThrCysGlnThrGlyGlnThrSerLeuSerAspProValHisLeuThrValLeuSerGluTrpLeuValLeuGlnThrProHisLeuGluPhe
GCCAACAACAATGACAGCGGGGAGTACACGTGCCAGACTGGCCAGACCAGCCTCAGCGACCCTGTGCATCTGACaGTGCTTTCTGAGTGGCTGGTGCTCCAGACCCCTCACCTGGAGTTC

                    110                                                                                             140     *
GlnGluGlyGluThrIleValLeuArgCysHisSerTrpLysAspLysProLeuValLysValThrPhePheGlnAsnGlyLysSerLysLysPheSerArgSerAspProAsnPheSer
CAGGAGGGAGAAACCATCGTGCTGAGGTGCCACAGCTGGAAGGACAAGCCTCTGGTCAAGGTCACATTCTTCCAGAATGGAAAATCCAAGAAATTTTCCCGTTCGGATCCCAACTTCTCC

                                                                                                    170
IleProGlnAlaAsnHisSerHisSerGlyAspTyrHisCysThrGlyAsnIleGlyTyrThrLeuTyrSerSerLysProValThrIleThrValGlnAlaProSerSerSerProMet
ATCCCACAAGCAAACCACAGTCACAGTGGTGATTACCATTGCACAGGAAACATAGGCTACACGCTGTACTCATCCAAGCCTGTGACCATCACTGTCCAAGCTCCCAGCTCTTCACCGATG

                                                  200
GlyIleIleValAlaValValThrGlyIleAlaValAlaAlaIleValAlaAlaValValAlaLeuIleTyrCysArgLysLysArgIleSerAlaAsnSerThrAspProValLysAla
GGGATCATTGTGGCTGTGGTCACTGGGATTGCTGTAGCtGCCATTGTTGCTGCTGTAGTGGCCTTGATCTACTGCAGGAAAAAGCGGATTTCAGCCAATTCCACTGATCCTGTGAAGGCT

                    230                                                                                             260
AlaGlnPheGluProProGlyArgGlnMetIleAlaIleArgLysArgGlnProGluGluThrAsnAsnAspTyrGluThrAlaAspGlyGlyTyrMetThrLeuAsnProArgAlaPro
GCCCAATTTGAGCCACCTGGACGTCAAATGATTGCCATCAGAAAGAGACAACCTGAAGAAACCAACAATGACTATGAAACAGCTGACGGCGGCTACATGACTCTGAACCCCAGGGCACCT
                                                                                                                ::::::::::::::::::::::::::::::::::::::::

ThrAspAspAspLysAsnIleTyrLeuThrLeuProProAsnAspHisValAsnSerAsnAsnEnd
ACTGACGATGATAAAAACATCTACTTGACTCTTCCTCCCAACGACCATGTCAACAGTAATAACTAAGAGTAACGTTATGCCATGTGGTCACCTCTCAGCTTGCTGATGGATGACAAAAAG
AGGGGAATTGTTAAAGGAAAATTTAAATGGAGACTGGAAAAATTCCTGAGCAAACAAAACCACCTGGCCCTTAGAAATAGCTTTAACTTTGCTTAAACTACAAACACAAGCAAAACTTCA
CGGGGTCATACTACATACAAGCATAAGCAAAACTTAACTTGGATCATTTCTGGTAAATGCTTATGTTAGAAATAAGACAACCCCAGCCAATCACAAGCAGCCTACTAACATATAATTAGG
TGGCTAGGGACTTTCTAAGAAGATACCTACCCCCAAAAAACAATTATATAATTGAAAACCAATCGATTGCCTTTATTTTGCTTCCACATTTTCCCAATAAATACTTGCCTGTGACATTTT
GCCACTGGAACACTAAAAAAAA(A)n
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Fig. 1. The complete nucleotide sequence and deduced amino acid sequence for hFcRII-B. There are two potential ATG starts sites indicated by −42 and −15 above the proposed signal peptide. The proposed amino terminus of the mature FcRII molecule following signal peptide cleavage is indicated by '+1' over Thr; this site was predicted on the basis of sequence homology with mFcRII, for which amino-terminal sequence information has been obtained (Lewis *et al.*, 1986; see Figure 3). The ectodomain consists of 175 amino acid residues and contains four evenly spaced Cys residues (bold) whose surrounding sequences are related to other members of the immunoglobulin gene superfamily (Williams and Barclay, 1988). It also contains three potential sites for N-linked glycosylation (Asn-X-Ser; X ≠ Pro) (bold and underlined; the second glycosylation site has been marked with an asterisk (*) since it is absent from the hFcRII-A sequence but present in mFcRII (see Figure 3). A single hydrophobic domain 27−29 amino acid residues in length presumably indicates the membrane-spanning domain (overlined); the precise start of this domain is ambiguous, however, due to the absence of highly charged residues prior to the membrane insertion site. The hydrophobic segment is followed by a charged stop-transfer sequence (Arg-Lys-Lys-Arg) and then by the presumptive cytoplasmic tail, 76 amino acids in length. There is a polyadenylation signal, AATAAA (underlined), in the 3′ UT region. Black dots before the start of the UT region indicate the start of the 600 bp *Pvu*II−*Eco*RI restriction fragment used to prepare a unique hFcRII-A/B-specific cDNA probe. The probe terminated at the 3′ end of the cDNA.

isoform identified thus far is up to 70% similar to FcRII-B1, -B2 or -A in its extracellular and/or membrane-spanning domains, its cytoplasmic tail is completely different from any previously described murine FcR (Stuart *et al.*, 1987). Since the sequence of the murine FcRII cytoplasmic domains are clearly important in such functions as endocytosis (Miettinen *et al.*, 1989), the possible occurrence of similar cytoplasmic domain heterogeneity within the human FcRII class is of particular interest.

Comparatively little is known concerning the structure and function of IgG FcR on epithelial cells. However, there are indications that epithelial FcR may bear an unexpected functional relationship to FcR of the immune system. Expression of mouse FcR in polarized Madin−Darby canine kidney cells has demonstrated that mFcRII-B2 can mediate the efficient apical to basolateral transcytosis of IgG (Hunziker and Mellman, 1989). While possibly fortuitous, this is the expected direction of transcellular transport of 'authentic' epithelial IgG FcR in neonatal rat intestine and placental syncytiotrophoblasts (Mellman *et al.*, 1988). Structurally, neither human nor mouse FcRII is related to the intestinal receptor, which has recently been found to be an MHC class I-like molecule, and thus, a member of the immunoglobulin gene superfamily (Simister and Mostov, 1989). A relationship to the syncytiotrophoblast FcR is possible, however, since the human placental receptor is thought to have a mol. wt (40−45 kd) (Matre *et al.*, 1981) similar to that of hFcRII.

In this paper, we sought to identify additional hFcRII isoforms and to investigate further the possible relationship between IgG FcR of the immune system and epithelia. Two novel full-length hFcRII cDNA clones were isolated from a human placental cDNA library. While the first of these cDNA clones (designated hFcRII-B) was closely related to but distinct from the human FcRII cDNA clone described previously (hFcRII-A) (Stuart *et al.*, 1987), the second (hFcRII-C) was highly homologous to the murine macrophage isoform mFcRII-B2. While all three isoforms were found in a variety of monocytic and lymphoid cells, transcripts and antigen identical or related to hFcRII-C were also expressed in placental syncytiotrophoblasts. Combined with the evidence that the murine homologue of hFcRII-C can mediate transcytosis when transfected into an epithelial cell line, these results suggest that FcR found in the immune system and in certain transporting epithelia may be functionally and structurally related.

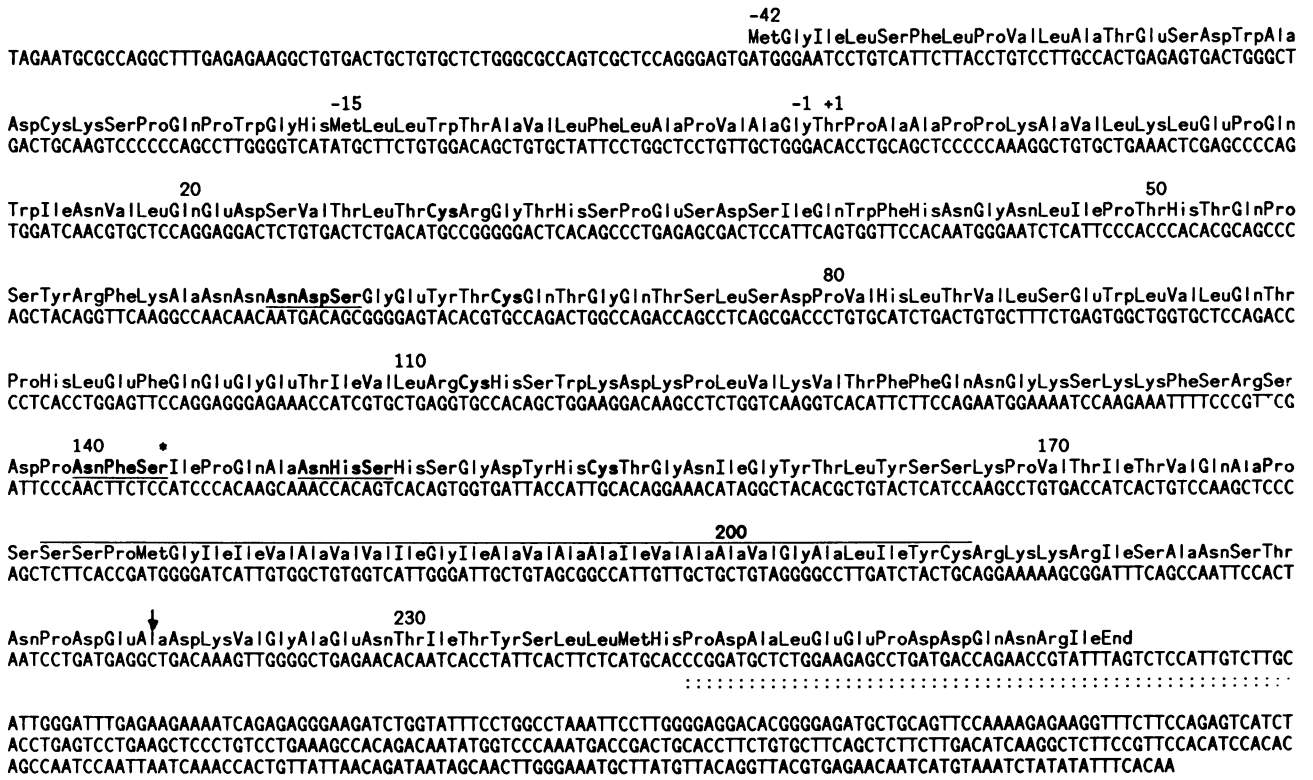

Fig. 2. The nucleotide and deduced amino acid sequence for the third human FcRII isoform, hFcRII-C. The signal sequence and extracellular domains predicted by the hFcRII-C cDNA are identical to the corresponding regions described for hFcRII-B in the legend to Figure 1. The ectodomain consists of 175 amino acid residues, contains four evenly spaced cysteine residues (bold) and three potential N-linked carbohydrate addition sites (bold and underlined). The transmembrane segment consists of 27−29 highly hydrophobic amino acid residues (overlined) and terminates with the same stop transfer sequence described for hFcRII-A and -B. The hydrophilic cytoplasmic domain consists of 44 amino acid residues; where this domain diverges from either hFcRII-A or -B has been indicated by an arrow preceding Asp_{220}. Since a polyadenylation signal was not found in the 3′ UT region of this cDNA, it is presumably incomplete. Black dots indicate the start of the 400 bp *Hpa*II−*Eco*RI restriction fragment used to prepare an hFcRII-C-specific cDNA probe which terminated at the end of the cDNA. The probe was used for both Northern blots and *in situ* hybridization.

Results

Two unique cDNAs from human placenta encode novel structures for hFcRII

Using a cDNA probe representing the entire open reading frame of the murine FcRII-B2 (Lewis *et al.*, 1986), 40 clones were isolated from a λgt11 placenta cDNA library which cross-hybridized under reduced stringency conditions at a frequency of ~0.04%. Two cDNAs that exhibited distinct restriction maps were sequenced on both strands using the dideoxy method. The complete nucleotide and predicted protein sequences of clone 1, designated hFcRII-B, are shown in Figure 1; those for clone 2, designated hFcRII-C, are in Figure 2.

The two new hFcRII cDNAs are closely related to but distinct from the previously isolated hFcRII-A cDNA (Stuart *et al.*, 1987; Hibbs *et al.*, 1988; Stengelin *et al.*, 1988). Although the predicted amino-terminal signal sequence of hFcRII-A is unrelated to the corresponding region shared by both hFcRII-B and -C, the ectodomains of all three sequences exhibit a high degree of similarity. hFcRII-B and -C are identical in this region and differ from hFcRII-A only by eight scattered amino acid substitutions (96% identity overall at the protein level) (Figure 3). Among these differences is the presence of only two potential sites for N-linked glycosylation (Asn-X-Ser/Thr; X ≠ Pro; underlined in Figure 3) in hFcRII-A while both hFcRII-B and -C contain a third site. The position of the third site, at Asn_{140}, was lost from hFcRII-A by a single amino acid change (Asn to Thr). It is homologous to one of four glycosylation sites found in murine FcRII sequences, all of which are used (Green *et al.*, 1985; Lewis *et al.*, 1986; Stuart *et al.*, 1987; Mellman, 1989). Finally, in both hFcRII-B and -C, the region of the ectodomain just before the predicted membrane insertion site is missing three amino acid residues compared to the sequences of either hFcRII-A or any of the murine FcRII sequences (Figure 3). The subequent membrane-spanning domains of all three hFcRII sequences are nearly identical.

The cytoplasmic tails of hFcRII-A and hFcRII-B are both 76 amino acids in length and were found to exhibit only a single amino acid residue difference between them (Leu to Pro at position 298 in hFcRII-B; Figure 3). This difference appears not to be a cloning artefact since multiple, independently isolated cDNA clones exhibited the same two sequences (not shown). The homology between hFcRII-A and -B extends throughout the 3′ untranslated region encoded by both cDNAs (see Figures 1 and 2). The cytoplasmic domain of hFcRII-C, in contrast, is comparatively unrelated to the corresponding regions of hFcRII-A or -B, exhibiting <30% homology at the amino acid level. Much of this similarity is limited to the sequence most proximal to the likely membrane insertion site: although the first nine amino acid residues of all three cytoplasmic domains are identical,

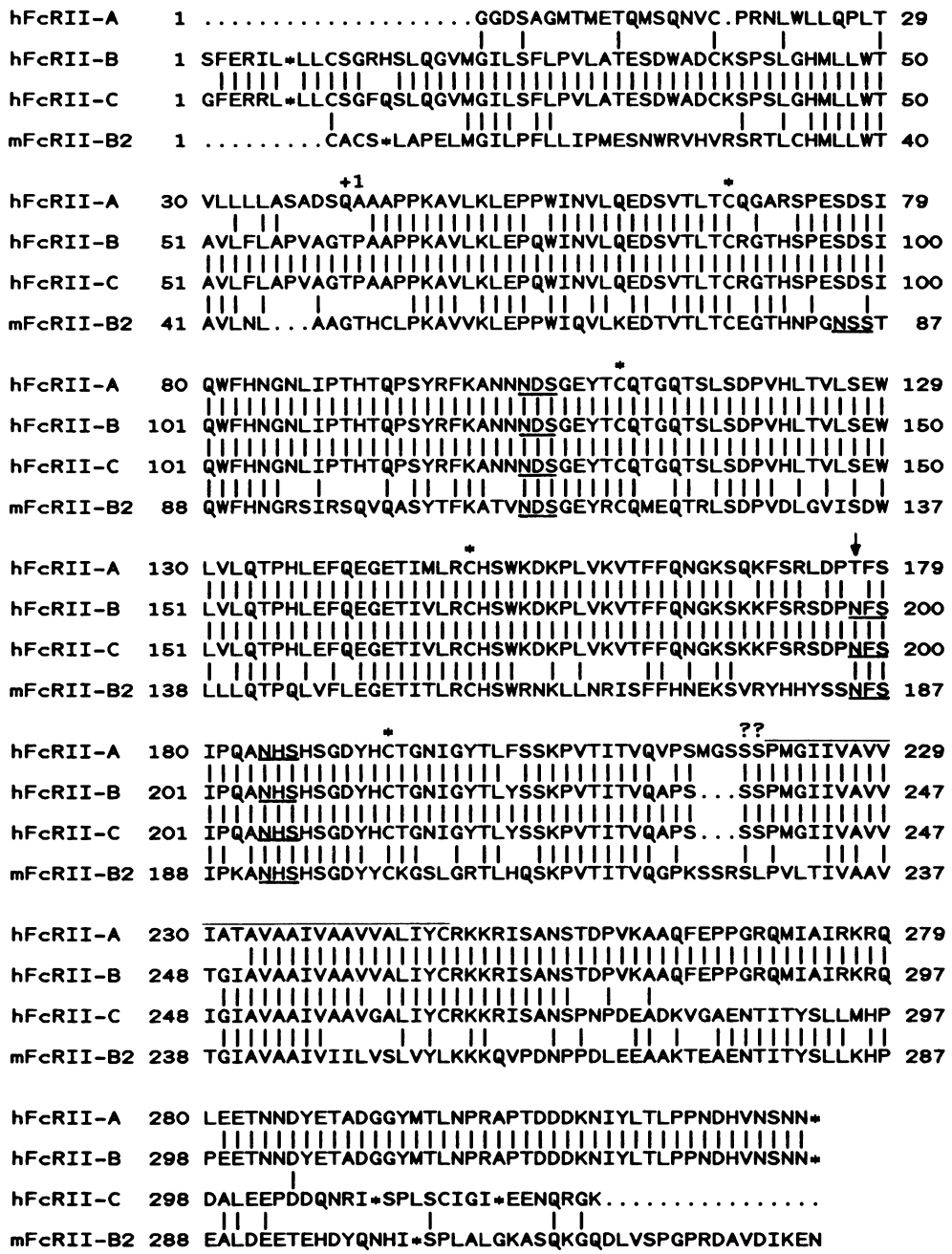

Fig. 3. Amino acid sequence comparison of human FcRII-A, -B, -C and murine FcRII-B2. Vertical lines indicate positions of amino acid identity. Asterisks (*) denote the positions of the four evenly spaced cysteine residues whose relative positions are conserved in the ectodomains of all four molecules. Potential sites for Asn-linked glycosylation are underlined, with the arrow indicating a glycosylation site in hFcRII-B and -C which is absent from hFcRII-A. This site is found in all murine FcRII sequences. The probable start of the membrane-spanning domain of the three human sequences is somewhat ambiguous, and is indicated by question marks (??) on the basis of sequence homology with murine FcRII; the membrane-spanning domain itself is overlined. While the four sequences have highly homologous ectodomains and membrane-spanning segments, their predicted signal sequences and cytoplasmic tails diverge considerably. Of the human receptor isoforms, only hFcRII-C has a cytoplasmic tail homologous to mFcRII-B2. This homology is most extensive within a region of the cytoplasmic tail that may be involved in coated pit localization (Miettinen *et al.*, 1989).

there is no significant homology observed between hFcRII-C and -A/-B in the distal portion of the tail. However, the cytoplasmic domain as well as the 3′ untranslated sequence of the hFcRII-C are closely related to the corresponding region of the murine FcRII-B2 isoform, expressed primarily by macrophages (>60% identity at both nucleotide and amino acid levels). The homology was greatest at a region of the cytoplasmic tail that may be necessary for mFcRII-B2 localization at clathrin-coated pits (Miettinen *et al.*, 1989). In contrast, there was no homology between hFcRII-C and the insertion of 47 amino acids found in the cytoplasmic tail of the alternatively spliced murine isoform designated mFcRII-B1 that is expressed predominantly in B-lymphocytes (Hogarth *et al.*, 1987; Mellman, 1989).

Taken together, the sequence data indicate that human FcRII exists as a family of multiple isoforms. Although some of the amino acid substitutions may be explained by allelic variation, it seems likely that the receptors are derived from at least two alternatively spliced genes, as is thought for the homologous family of murine FcRII (Mellman, 1989). As

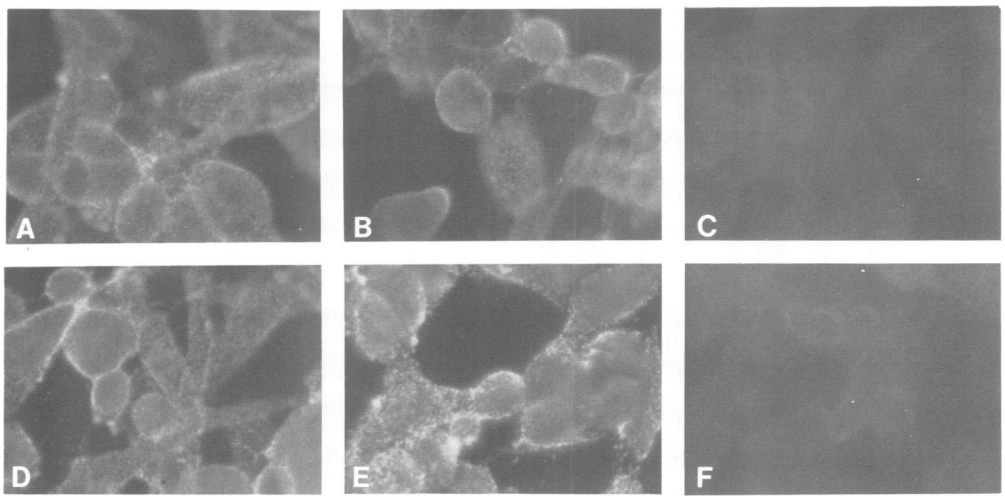

Fig. 4. Immunofluorescence of hFcRII-B and hFcRII-C in transfected CHO cells. Chinese hamster ovary (CHO) cells were transfected with cDNAs encoding hFcRII-B (top three panels) or hFcRII-C (bottom three panels) using the pFRSV expression vector. Cells were plated on glass coverslips and surface expression of the two hFcRII isoforms was characterized by indirect immunofluorescence. (**Panels A** and **D**) Unfixed cells incubated with heat-aggregated human IgG (1 h, 4°C) followed by $F(ab')_2$ fragments of FITC-conjugated goat anti-human IgG. (**Panels B** and **E**) Unfixed cells stained with the anti-hFcRII monoclonal antibody IV.3 followed by FITC-$F(ab')_2$ anti-mouse IgG; identical results were obtained using a second anti-hFcRII monoclonal antibody, KuFc79. (**Panel C**) Unfixed cells stained with a human myeloma IgA (1 h, 4°C), followed by FITC-$F(ab')_2$ anti-human IgA. (**Panel F**) hFcRII-C transfected cells stained with a non-specific murine myeloma IgG2b, followed by FITC-$F(ab')_2$ anti-mouse IgG.

for mFcRII isoforms, the greatest degree of variation among the human receptors is evident in their respective cytoplasmic domains.

hFcRII cDNAs direct cell surface expression of isotype-specific FcR

hFcRII-B and hFcRII-C were subcloned into an SV40-based expression vector, pFRSV (Horwich *et al.*, 1985; Miettinen *et al.*, 1989) and expressed transiently in mouse L cells and permanently in Chinese hamster ovary (CHO) cells. As shown by immunofluorescence, both cDNAs directed expression of a surface protein which bound heat-aggregated human IgG (Figure 4, panels A and D) but not human myeloma IgA (Figure 4, panel C). Two monoclonal antibodies, IV.3 (Looney *et al.*, 1986) and KuFc79 (Vaughn *et al.*, 1985), which recognize different surface epitopes associated with human FcRII, also bound cells transfected with hFcRII-B (Figure 4, panel B) and hFcRII-C (Figure 4, panel E) cDNAs, as previously found for hFcRII-A (Stuart *et al.*, 1987). No staining was observed using isotype-matched, non-specific control monoclonal antibodies (panel F) or when hFcRII-B or -C cDNA was inserted in the expression vector in the antisense orientation. Thus, all three human FcR cDNAs encode FcRs exhibiting ligand binding and monoclonal antibody reactivity characteristic of hFcRII (Anderson and Looney, 1986).

Expression of hFcRII mRNA in monocytic and lymphoid cell types

To determine if the different human FcRII isoforms may be associated with unique functions, we first investigated their cellular expression by Northern blot analysis. Expression of hFcRII-C was distinguished from expression of hFcRII-A or -B using probes derived from restriction fragments corresponding to the unique portions of their cytoplasmic domains. hFcRII-A and -B were too closely related to be distinguished in this way.

Previous Northern blot analysis of FcRII-positive human cell types with full-length hFcRII-A cDNA consistently showed two transcripts of 1.6 and 2.5 kb (Stuart *et al.*, 1987). The longer transcript represented a second polyadenylated form of hFcRII mRNA which contained an additional 900 bp of 3′ untranslated (UT) sequence (Hibbs *et al.*, 1988; Stengelin *et al.*, 1988). The additional sequences exhibited ~90% nucleotide homology to the 3′ half of the coding region (cytoplasmic tail) and 3′ UT of hFcRII-C. Since the 2.5 kb hFcRII-A would thus be recognized by the hFcRII-C cytoplasmic tail probe, it is possible that only the 1.6 kb mRNA represents the authentic hFcRII-C transcript.

Northern blot analysis was performed using total RNA isolated from a variety of human lymphoid and myeloid cell lines, as well as from primary human alveolar macrophages. To determine whether one of the hFcRII cDNAs might detect a related FcR species in the IgG-transporting epithelium of

Table I. Expression (Northern blot analysis) of human FcRII isoforms in different human cell types

Cell type	Probe[a]			
	hFcRII-A/-B		hFcRII-C	
	2.5 kb[b]	1.6 kb	2.5 kb	1.6 kb
(A) Monocyte/macrophage				
U937	++	+	++	+
HL-60	+	−	−	−
K562	++	++	−	−
Alveolar macrophage	+++	++	++	+
(B) B-cell linese				
Daudi	+	+	+	++
Raji	+	−	+	+
(C) T-cell line				
CEM	−	−	−	−
(D) Epithelium (trophoblasts)	++	+	++++	++
(E) Fibroblast (HeLa)	−	−	−	−

[a]Restriction fragments representing the unique cytoplasmic domains for hFcRII-A/B or hFcRII-C, see legend to Figures 1 and 2.
[b]The two transcripts represent different polyadenylated forms encoding hFcRII (Stengelin *et al.*, 1988; see text for details).

the placenta, we also analyzed RNA from primary cultures of trophoblasts isolated from human term placenta by trypsin/DNase digestion and Percoll gradient centrifugation (Kliman *et al.*, 1986). The results from these experiments are summarized in Table I, with a representative Northern blot shown in Figure 5.

There appeared to be little consistent differential expression of hFcRII transcripts among the cells of myeloid or lymphoid origin. Either or both the 1.6 and 2.5 kb transcripts were found in all FcR-positive cells and cell lines analyzed using cDNA probes specific for hFcRII-A/-B or hFcRII-C, including primary alveolar macrophages (Figure 5; Table I). Thus, unlike the case for murine FcRII-A and -B2 transcripts (Lewis *et al.*, 1986; Ravetch *et al.*, 1986; T.Koch *et al.*, in preparation), there did not appear to be a clearly defined 'monocyte-specific' human FcRII. Only K562 and HL-60 promyelocytes expressed hFcRII-A/-B mRNA without containing detectable hFcRII-C message. Expression in Daudi cells, a B-cell line derived from a Burkitt's lymphoma, exhibited marked clonal variation with at least one Daudi subclone showing no mRNA encoding any of the hFcRII isoforms. No hybridization was found in RNA from FcR-negative CEM T-cells or HeLa cells (Table I).

hFcRII-C is expressed in placental syncytiotrophoblasts

More remarkable than the absence of differential hFcRII expression among diverse FcR-positive hematopoietic cells was the presence of transcripts encoding hFcRII in unrelated epithelial cells. As shown in Figure 5, RNA extracted from placental trophoblast cultures exhibited the 2.5 and 1.6 kb transcripts using both hFcRII-A/-B and hFcRII-C probes. In contrast to the results obtained with all other cells analyzed, however, the 2.5 kb message was found to hybridize far better with the hFcRII-C probe than with the hFcRII-A/-B probe. Thus, it is likely that much of the 2.5 kb transcript in trophoblasts reflected hFcRII-C mRNA, as opposed to hFcRII-A mRNA containing hFcRII-C-related sequences in its 3′ UT region (Stengelin *et al.*, 1988; see above). Similarly, this result argues against the possibility that the presence of hFcRII-C transcripts in trophoblast RNA was due entirely to contamination by tissue macrophages (Hofbauer cells): hybridization obtained using the hFcRII-A/-B-specific probe in monocytic cells (e.g. alveolar macrophages, U937 cells) always exceeded that obtained using the hFcRII-C probe (Figure 5).

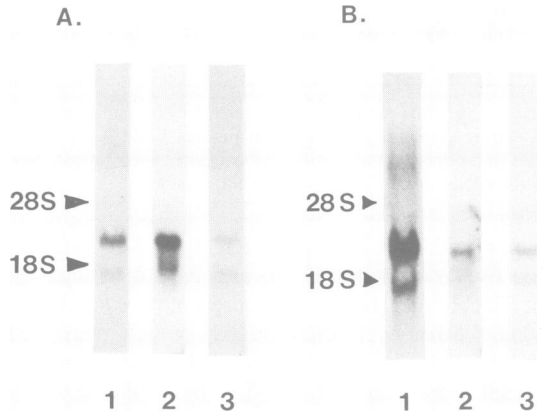

Fig. 5. Northern blot analysis of hFcRII expression in human cell types. Fifteen micrograms of total RNA from human placental trophoblast cultures (**lane 1**), human alveolar macrophages (**lane 2**) and the promyelocytic cell line U937 (**lane 3**) were electrophoresed in formaldehyde/agarose gels and transferred to Gene Screen Plus according to the manufacturer's specifications. In **panel A**, RNAs were hybridized to a cytoplasmic tail probe for hFcRII-A and -B; in **panel B**, RNAs were hybridized using the cytoplasmic tail probe for hFcRII-C (see text for details). All lanes were exposed for 48 h, except for alveolar macrophage RNA in lanes 2 which were exposed for 20 h.

To investigate more directly the expression of hFcRII-C transcripts by placental epithelium, we next performed *in situ* hybridization and immunohistochemistry using sections of human hydatidiform moles, a placental neoplasm with marked proliferation of invasive syncytiotrophoblasts relative to term placenta. For *in situ* hybridization, surgically removed moles were immediately fixed in paraformaldehyde−glutaraldehyde (4−0.5%), embedded in paraffin, and then sectioned. Hybridization was performed using a ^{35}S-labeled restriction fragment corresponding to the unique cytoplasmic tail of hFcRII-C. For a positive control, sections were also hybridized with a probe for another monocyte marker, the c-*fms* proto-oncogene [colony stimulating factor-1 (CSF-1) receptor] whose expression has previously been localized to syncytiotrophoblasts in human and mouse placenta (Hoshina *et al.*, 1985; Rettenmeier *et al.*, 1986; Pollard *et al.*, 1987, 1989; Arceci *et al.*, 1989) and β-actin (which should be expressed in all cells of the villus). Background levels of non-specific probe binding were determined using sections hybridized with β-lactamase cDNA probe.

As shown in Figure 6, silver grains were concentrated over histologically identifiable syncytiotrophoblasts lining placental villi using both the hFcRII-C (cytoplasmic tail) (panel A) and c-*fms* (panel B) probes. Lower levels of hybridization were detected over areas of cytotrophoblast differentiation while only background levels (similar to those obtained using β-lactamase as a probe; panel C) were seen over the connective tissue stroma within the villi, or in areas of the sections devoid of tissue. As expected, when the β-actin probe was used (to detect a constitutively expressed gene), a more homogenous distribution of grains was observed over all tissue-containing areas of the section (not shown).

To quantify these results, the number of grains localized over syncytiotrophoblasts was determined using a computer-assisted optical grain counter to yield counts per μm^2. Data were obtained for 10−12 separate 100× fields following hybridization with probes for hFcRII-C, c-*fms*, β-actin or β-lactamase. Hybridization for each probe (in hybrids per μm^2) was determined from the grain count values after accounting for relative probe length, specific activity, length of exposure, section thickness and non-specific background binding. As summarized in Table II, hybrid levels over syncytiotrophoblasts due to the hFcRII-C probe were 5- to 10-fold higher than those observed for either β-actin or c-*fms* probes.

To detect the expression of hFcRII at the protein level, immunohistochemistry was performed on sections of formalin-fixed hydatidiform moles. Sections were incubated with the anti-hFcRII monoclonal antibody IV.3 IgG (Looney *et al.*, 1986) or non-specific total mouse IgG, and antibody binding visualized using biotin-conjugated second antibodies/streptavidin−horseradish peroxidase (HRP) and diaminobenzidine (DAB). As shown in Figure 7, HRP−DAB reaction product was clearly seen associated with histologically identifiable syncytiotrophoblasts irrespective of

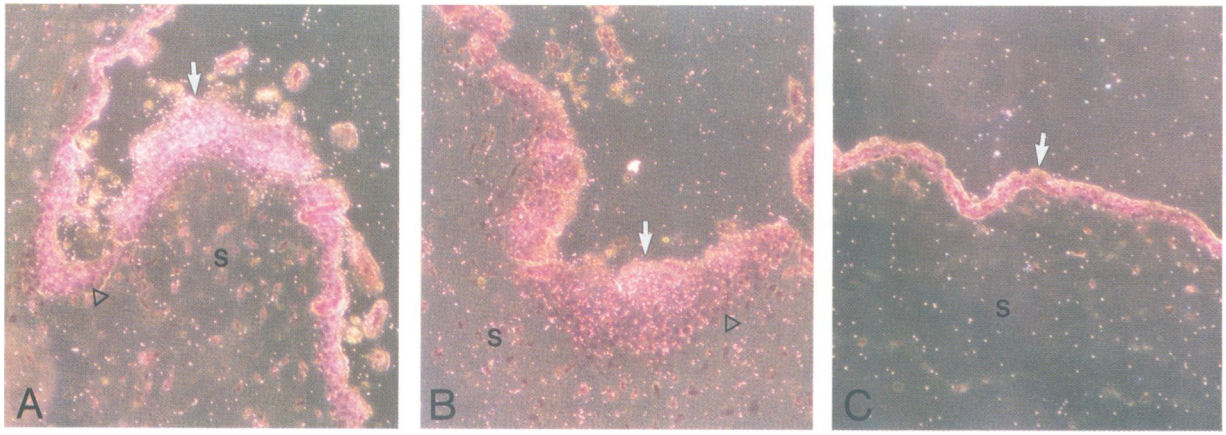

Fig. 6. Expression of hFcRII-C, c-*fms* and β-actin mRNA in human placental tissue using *in situ* hybridization. Sections of human hydatidiform moles were hybridized with ^{35}S-labeled cDNA probes, autoradiographed, stained with hematoxylin and eosin, and then viewed by dark-field microscopy using a dark-field condensor (Olympus). (**Panel A**) hFcRII-C: most of the grains are localized over the syncytiotrophoblasts on the external surface of the chorionic villus (white arrow), with less hybridization over areas containing less syncytiotrophoblast and more cytotrophoblast (open arrowhead) or over the connective tissue stroma (S) within the villus. (**Panel B**) c-*fms* CSF-1 receptor: hybridization using a c-*fms* probe was restricted largely to areas of prominent syncytiotrophoblasts (white arrow). (**Panel C**) β-Lactamase: these sections reflect the degree of non-specific hybridization to tissue and non-tissue areas of the specimens.

Table II. Quantitative analysis of *in situ* hybridization of human placental syncytial trophoblasts

	hFcRII-C	c-*fms*	β-Actin	β-Lactamase[a]
Hybrids (μm^2	1.43×10^{-2}	0.36×10^{-2}	2.0×10^{-3}	0
Standard error/μm^2	0.05×10^{-2}	0.01×10^{-2}	$<0.01 \times 10^{-2}$	0

In situ hybridization was performed on sections of human hydatidiform moles as described in the text. Grains localized by dark- and light-field microscopy to areas of prominent syncytiotrophoblasts were scored using a computer-assisted optical grain counter. The resultant grains per square micron values were converted to hybrids per square micron by multiplicative factors which take into account probe length, specific activity and length of autoradiographic exposure. For both the hFcRII-C or c-*fms* probes, grain counts over cytotrophoblasts, connective tissue stroma, or areas of the section devoid of tissue were not significantly different from the β-lactamase negative control.
[a]Counts per square micron for the β-lactamase probe were subtracted from all other values.

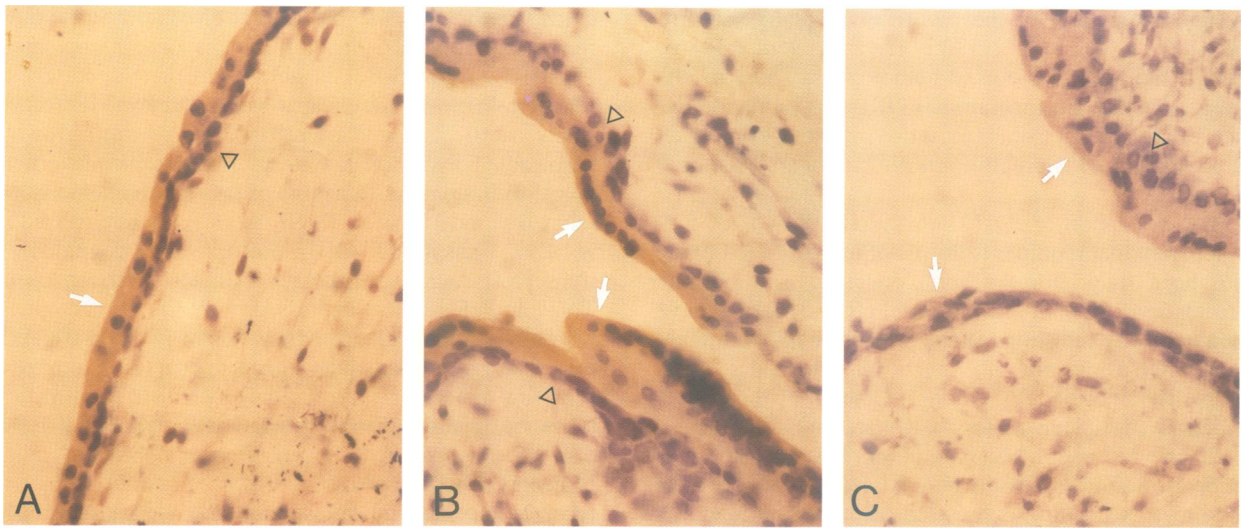

Fig. 7. Detection of hFcRII expression in human placental syncytiotrophoblasts by immunohistochemistry. Sections of human hydatidiform moles were incubated with similar dilutions of the mouse anti-hFcRII monoclonal antibody IV.3 (**panels A** and **B**) or a non-specific total mouse IgG (**panel C**). Bound antibody was then visualized using biotin-coupled second antibody, horseradish peroxidase-coupled streptavidin and diaminobenzidine$-H_2O_2$. The brown diaminobenzidine reaction product was clearly visible in syncytiotrophoblasts (white arrows), whether the cells were present as a single (**panel A**) or multi-cellular (**panel B**) epithelial sheet. No staining was visible in the underlying cytotrophoblast layer (marked with open arrowheads where visible) and only occasional cells were stained within the connective tissue stroma. No reaction product was seen in sections incubated with the control IgG (**panel C**). Sections were stained with hematoxylin prior to microscopy, and photographed with a 20× objective using bright-field optics and Kodak Ektachrome 160 and printed using Cibachrome paper.

whether the cells were present as single (panel A) or multilayered (panel B) epithelial sheets. As expected, similar results (not shown) were obtained using an antibody to the CSF-1 receptor, previously demonstrated to be expressed in syncytiotrophoblasts (Rettenmeier *et al.*, 1986; Arceci *et al.*, 1989). In contrast, no staining was observed in sections reacted with control IgGs (Figure 7C).

Taken together, the Northern blot, *in situ* hybridization and immunohistochemical experiments suggest that placental syncytiotrophoblasts express a receptor similar or identical to the macrophage-lymphocyte hFcRII-C isoform. Conceivably, this FcR may play an unexpected role in the transcytosis of maternal IgG to the fetal circulation, a function supported by recent experiments using transfected MDCK cells (Hunziker and Mellman, 1989).

Discussion

We have isolated and characterized full-length cDNAs encoding two new hFcRII isoforms, designated hFcRII-B and -C, from a human placenta library. Alhough closely related, the nucleotide and predicted amino acid sequences of these receptors are clearly distinct from the only other previously characterized isoform, hFcRII-A (Stuart *et al.*, 1987; Hibbs *et al.*, 1988; Stengelin *et al.*, 1988). The receptor encoded by the hFcRII-B cDNA may be identical to a partial cDNA isolated by Stengelin *et al.* (1988), although the ectodomain predicted by this clone was interrupted by a small intron and ended after only nine amino acids of the cytoplasmic domain. hFcRII-C is a new sequence which encodes a third human hFcRII. While hFcRII-A and -B are closely related throughout their lengths, hFcRII-C is significantly different. Although its ectodomain and membrane-spanning region are nearly identical to hFcRII-A and -B, hFcRII-C diverges after the first 12 amino acid residues of its cytoplasmic tail (Figure 3). On the other hand, the endodomain of hFcRII-C is >60% identical to the corresponding region of the mouse macrophage isoform mFcRII-B2 (Lewis *et al.*, 1986; Ravetch *et al.*, 1986), making it and hFcRII-C closest homologues. These results suggest, as is thought for murine FcRII, that human FcRII sequences are derived from at least two separate genes which may be subject to alternative mRNA splicing (Mellman, 1989).

With the isolation of cDNAs for hFcRII-B and -C, it is now apparent that multiple isoforms of human FcRII exist, as has been found for the homologous class of murine Fc receptors. In both cases, the FcRII isoforms have immunologically indistinguishable ectodomains, but highly variable cytoplasmic tails. The functional significance of this heterogeneity, particularly among members of the human FcRII family, is unknown. Thus far, there is no clear pattern of cell type restriction that would implicate a particular functional role for any of the human isoforms. In the case of the mouse receptors, expression of individual isoforms has not only been associated with particular cell types, but studies of receptor-negative cells transfected with mFcRII cDNAs have also demonstrated that distinct functional differences exist. mFcRII-B1 and -B2, for example, differ greatly in their abilities for receptor-mediated endocytosis since only mFcRII-B2 is capable of accumulating at clathrin-coated pits on the plasma membrane (Miettinen *et al.*, 1989). The two isoforms differ only in their cytoplasmic tails. mFcRII-B2 is expressed by macrophages, a cell type well known for FcR-mediated endocytosis, while mFcRII-B1 is expressed predominantly by B-lymphocytes which do not mediate the uptake of FcR-bound ligands (H.Miettinen *et al.*, unpublished) or present antigen via this receptor (Kehry and Yamashita, 1989). It seems likely that similar functional heterogeneity will also be found among human FcRII isoforms.

In this respect, Northern blot, *in situ* hybridization and immunohistochemical results have already suggested at least one unexpected possible function of hFcRII-C: transepithelial transport of maternal IgG. While almost all FcRII-positive human cells and cell lines of lymphoid and myeloid origin tested expressed mRNA for hFcRII-A, -B and -C, significant expression—particularly of hFcRII-C mRNA—was found in placental syncytiotrophoblasts, the cells comprising the IgG-transporting epithelium of chorionic villi. Although existing antibodies to hFcRII do not distinguish among the three known receptor isoforms (Figure 4; Stuart *et al.*, 1987), it is likely that the immunoreactive protein observed by immunohistochemistry reflects expression of hFcRII-C.

While syncytiotrophoblasts might express both hFcRII-C and a second FcR that is actually the receptor responsible for mediating transcytosis of maternal IgG, additional considerations support the possibility that the leukocyte and placental receptors are one and the same. First, previous efforts to isolate and characterize the placental FcR have yielded a 40 kd protein (Matre *et al.*, 1981) which is very similar in size to that of mature hFcRII (Looney *et al.*, 1986) and predicted by cDNAs. Second, mAb to the purified placental protein appears to react not only with placenta, but also with 3- to 4-day cultured monocytes and placental macrophages (Hofbauer cells) (R.Matre, personal communication).

In the placenta, syncytiotrophoblasts form an attenuated epithelial cell layer over the surfaces of the chorionic villi. In order for these cells to mediate the transport of maternal IgG, placental FcR must be capable of IgG transcytosis in the apical to basolateral direction. Surprisingly, recent experiments indicate that the mouse homologue of hFcRII-C, mFcRII-B2, is able to mediate precisely this function. When Madin–Darby canine kidney (MDCK) cells stably transfected with mFcRII-B2 cDNAs are grown on permeable polycarbonate Costar® filters, rapid and efficient apical-to-basolateral transport of IgG can be demonstrated (Hunziker and Mellman, 1989). While some internalized IgG is also transported to lysosomes and degraded, no transcytosis in the basolateral to apical direction occurs. Thus, an FcR isoform expressed primarily in macrophages whose functions include endocytosis of IgG and triggering the release of inflammatory agents appears also to have the requisite molecular information to mediate unidirectional transcytosis in polarized epithelial cells. If mFcRII-B2 can also be demonstrated in syncytiotrophoblasts of murine placenta, it will be possible directly to test the role of this receptor in conferring passive immunity via transport of IgG from the maternal to the fetal circulation at different stages of gestation.

On the other hand, expression of hFcRII-C in syncytiotrophoblasts may be unrelated to the transport of maternal IgG and instead reflect a generalized expression of monocytic markers in this epithelium. As shown previously (and in Figure 6), cytotrophoblasts and/or syncytiotrophoblasts express other 'monocyte-specific' markers including the CSF-1 receptor or c-*fms* proto-oncogene (Rettenmeier *et al.*,

1986; Arceci *et al.*, 1989). While the precise role of c-*fms* expression in these cells is unknown, it is thought to be related to the proliferation and/or invasion of the placental epithelium into the uterine wall. It is possible that the presence of other monocyte-macrophage markers, such as hFcRII, reflects the expression of a generalized phenotype or transcriptional program which is characteristic of invasive cells of immune and non-immune origin (Kacinski *et al.*, 1988).

While additional work will be required to prove a functional relationship between the placental and leukocyte FcRs, it is now clear that like their murine counterparts human FcRIIs exist in multiple isoforms that are characterized by large variations in their cytoplasmic domains. While in the mouse much of this variation appears to be the result of alternative mRNA splicing, the basis for the variation among the human FcRIIs is less certain. The fact that both human and mouse isoforms tend to diverge at similar sites suggests that splice junctions or intron−exon boundaries are conserved (Mellman, 1989). For example, hFcRII-A and -B diverge from hFcRII-C at position 12 in the cytoplasmic domain. Similarly, mFcRII-B1 and -B2 differ due to an in-frame insertion in the cytoplasmic tail of mFcRII-B1, six amino acids from the membrane insertion site. While it is likely that the existence of such cytoplasmic tail heterogeneity reflects functional heterogeneity among the various receptor isoforms, the finding that hFcRII-C may be expressed in both leukocytes and epithelial cells suggests that a single receptor may also be able to perform unexpectedly diverse functions depending entirely on the cell type in which it is expressed.

Materials and methods

cDNA cloning and sequencing

An oligo d(T)-primed λgt11 cDNA library (Young and Davis, 1983) from human term placenta was obtained from Clontech Laboratories (Palo Alto, CA). Approximately 1×10^6 recombinants were screened using an α-^{32}P-labeled restriction fragment corresponding to the entire coding region of the mouse FcRII-B2 cDNA clone pFcR13 (Lewis *et al.*, 1986). Hybridization of nitrocellulose filters was performed under reduced stringency conditions (30% formamide, 5 × SSC, 5 × Denhardt's, 0.5% SDS at 42°C; filters were washed in 2 × SSC, 0.1% SDS at 50°C). Forty independent phage isolates were identified by cross-hybridization, of which 10 were further analyzed by restriction endonuclease mapping. Two cDNAs, found to be >1 kb in length and to have restriction patterns different from a cDNA encoding hFcRII-A (Stuart *et al.*, 1987), were subcloned into M13mp18/19 and sequenced on both strands using the dideoxy method of Sanger. In addition to using the M13 universal sequencing primer, synthetic oligonucleotides (14−17 bases in length) corresponding to hFcRII-B and -C cDNAs were also used.

Northern blot analysis

Total RNA was isolated from human term placenta, Daudi, Raji, CEM, HeLa, K562, U937, HL-60, peripheral blood leukocytes, human alveolar macrophages and cultured placental trophoblasts by a modification of the method of Chirgwin *et al.* (1979). Poly(A) selection, when used, was performed by cycling the RNA over oligo d(T) cellulose (Type 3, Collaborative Research, Bedford, MA) according to the manufacturer's recommendations. RNAs were fractionated by electrophoresis in 1% agarose/formaldehyde gels and transferred to nylon membranes (Gene Screen Plus, NEN Research Products, Boston, MA; or Biotrans, Schwarz-Mann Biotech, Cambridge, MA) as described by the manufacturer. Hybridization was performed in 50% formamide, 1.0 M NaCl, 1% SDS at 42°C using different ^{32}P-labeled (Feinberg and Vogelstein, 1983) restriction fragments encoding the different cytoplasmic tail and 3′ UT regions of the hFcRII isoforms. hFcRII-A and -B were detected using a *Pvu*II−*Eco*RI restriction fragment of hFcRII-A (Figure 1); hFcRII-C was detected using a *Hpa*II−*Eco*RI fragment (Figure 2).

In situ hybridization

Hydatidiform moles were surgically removed by suction curettage and fixed in freshly prepared 4% paraformaldehyde, 0.5% glutaraldehyde, 0.1 M sodium phosphate (pH 7.5) within 1−2 min after removal. After fixation, the tissue was washed in 50 mM Tris (pH 7.5), dehydrated to 100% ethanol, embedded in paraffin, and cut into 6 μm sections which were then placed onto polylysine-coated glass slides. *In situ* hybridization was performed by a slight modification of standard methods (Lawrence and Singer, 1985) as previously described (Kacinski *et al.*, 1988) using [^{35}S]cDNA probes labeled by random primer extension with [α-^{35}S]dCTP yielding specific activities of $\sim 5 \times 10^8$ d.p.m./μg of DNA. hFcRII-C was detected using a cDNA probe containing sequences corresponding to the cytoplasmic tail of hFcRII-C and the 3′ UT region (0.4 kb; the 3′ *Hpa*II to *Eco*RI restriction fragment). Other probes used were to the coding regions of c-*fms* (1.3 kb; 5′ fragment) (Roussel *et al.*, 1987), β-actin (2.0 kb) (Cleveland *et al.*, 1984) and β-lactamase (0.7 kb). Following autoradiography, hematoxylin and eosin-stained sections were viewed by bright and dark field microscopy at 10−20×. Grain density was determined microscopically using the Olympus Cue-2 Vision image analysis system (Olympus Corp., Tokyo) for areas of histologically identifiable syncytiotrophoblasts or cytotrophoblasts. Specific counts per μm^2 were determined by subtracting the corresponding values obtained using the non-specific β-lactamase probe. These values were then converted into 'hybrids/μm^2' by taking into account the exposure time for each sample, probe specific activity and probe length (Kacinski *et al.*, 1988).

All tissue specimens were obtained from patients at the Hunter Radiation Therapy and OB/GYN clinics of the Yale University School of Medicine in accordance with Yale HIC protocol no. 3303.

Immunohistochemistry

Sections of hydatidiform moles were obtained and prepared as described above for *in situ* hybridization, except that tissue was fixed in buffered formalin without glutaraldehyde. Fc receptors were detected using culture supernatants of the mouse anti-hFcRII antibody IV.3 (the kind gift of Clark Anderson, Ohio State University) followed by biotin-conjugated anti-mouse secondary antibody and streptavidin-HRP according to the manufacturer's recommendations (Vector Laboratories). Negative controls were performed using a non-specific total mouse IgG.

Transfection of FcR cDNAs

cDNAs encoding hFcRII-B and hFcRII-C were subcloned into pFRSV, an SV40-based derivative of pFR400 (Horwich *et al.*, 1985) which contained the dominant selectable marker DHFR. Transient expression of these cDNAs in murine Ltk$^-$ cells was first monitored 48−72 h after transfection of DEAE−dextran precipitates, as described (Stuart *et al.*, 1987). Stable expression in CHO cells was obtained by transfection with precipitates of calcium phosphate (Graham and van der Eb, 1973) and 10 μg of plasmid DNA. The precipitates were left on the plates for 6 h, removed, and the cells glycerol shocked (20% glycerol/PBS) for 1 min. Transfected 10 cm plates were split 1:10 72 h following introduction of the cDNAs and cultured for 16 days in α-MEM (minus nucleosides and nucleotides) containing 10% dialyzed fetal bovine serum, 4 μg/ml folic acid and 0.2 μM methotrexate (added from a 55 mM stock solution) (Horwich *et al.*, 1985). Resistant cells were cloned using cloning cylinders, expanded and checked for reactivity with the anti-FcRII monoclonal antibody IV.3 by immunofluorescence.

Immunofluorescence

Surface expression of FcRs on unfixed murine Ltk$^-$ or CHO cells was detected using heat-aggregated human IgG and anti-FcRII mAbs, IV.3 (Looney *et al.*, 1986) and KuFc79 (Vaughn *et al.*, 1985) on ice as described (Stuart *et al.*, 1987). Reactivity with the different mAbs was monitored on a Zeiss photomicroscope II equipped with fluorescence optics.

Cells and antibodies

CHO cells and all myeloid and lymphoid cell lines were maintained in α-MEM supplemented with 10% FBS, 100 μM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. COS7 and mouse Ltk$^-$ fibroblasts were grown in DME containing 10% FBS and antibiotics. Primary human alveolar macrophages were obtained by bronchoalveolar lavage. Primary trophoblast cultures were obtained from human term placenta by trypsin-DNase dispersion and Percoll gradient centrifugation exactly as described (Kliman *et al.*, 1986). Anti-receptor monoclonal antibodies IV.3 (hFcRII) and 32 (hFcRI) were gifts from Clark Anderson and Paul Guyre; Ku79 was the gift of T.Mohanakumar. Human IgG was obtained from Jackson Immunoresearch (West Grove, PA) and human myeloma IgA was from Cooper Biomedical (Malvern, PA). Heat aggregation of IgGs was performed

as described in Stuart *et al.* (1987). FITC-conjugated, affinity-purified second antibodies were obtained as $F(ab')_2$ fragments from Tago (Burlingame, CA).

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