ISOLATION AND EXPRESSION OF cDNA CLONES ENCODING A HUMAN RECEPTOR FOR IgG (FcγRII)

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Receptors for the Fc region of IgG (FcγR)[1] are expressed in a number of hematopoietic cell types and play important roles in several immunological processes such as phagocytosis of opsonized particulate antigens (1), clearance of immune complexes (2, 3), antibody-dependent cellular cytotoxicity (4, 5), signalling the production of inflammatory mediators (6), and regulation of Ig synthesis (7, 8).

Receptors for human IgG have been partially characterized as at least three distinct molecular species (9), which are expressed on different but overlapping subsets of hematopoietic cells. These FcγR classes have been termed FcγRI, FcγRII, and FcγRlo (10), and have been distinguished on the basis of size, affinity for subclasses of human (11–13) and mouse (14–16) IgG, distribution of expression on various cell types, in vitro functionality such as mediation of anti-T3 T lymphocyte proliferation (16, 17), and recognition by mAbs (16, 18–21). FcγRI is a 70-kD receptor expressed on human monocytes that exhibits a relatively high affinity for monomeric IgG (10^8–10^9/M, reference 12). FcγRII is a 40-kD species present on monocytes, B cells, neutrophils, platelets, and eosinophils that has a much lower affinity for ligand than FcγRI, preferring aggregated IgG (14, 16). FcγRlo is detected as a broad band of 50–70 kD on NK cells, neutrophils, eosinophils, T cells, and macrophages (13, 19, 22); this receptor apparently also binds only aggregated ligand.

A thorough understanding of the structure and biologic function of human FcγR requires analysis and manipulation of genes that encode these molecules. In this report, we describe the identification and expression of cDNA clones encoding one class (FcγRII) of human FcγR, and have determined that this receptor is the human homologue of the mouse macrophage/lymphocyte

[1] Abbreviations used in this paper: FcγR, Fc receptor for IgG; N-CAM, neural cell adhesion molecule; poly-IgR, epithelial transport receptor for polymeric IgA and IgM.

Fc[γ2b/γ1]R (23, 24). The human FcγR cDNA clones have a novel structure consisting of segments homologous to both the α and β mouse FcγR cDNAs (25, 26). While the two receptors are up to 61% homologous in their extracellular and membrane-spanning domains, they have completely unrelated cytoplasmic domains, a surprising observation for molecules that presumably mediate similar functions in human and mouse cells.

Materials and Methods

Cell Lines and Antibodies. U937 and HL-60 cells were provided by Dr. M. Siegel, Schering Corporation, Bloomfield, NJ. Daudi cells were provided by Dr. J. Banchereau, UNICET, Lyon, France. 166A2 cells were obtained from Dr. K. Ishizaka, The Johns Hopkins University, Baltimore, MD. Ltk⁻ cells were obtained from Dr. F. Lee, DNAX Research Institute. Culture supernatants containing mAbs 32 and IV.3 were kindly provided by Dr. C. Anderson, The Ohio State University, Columbus, OH. Leu-11b was purchased from Becton Dickinson & Co. (Mountain View, CA), and mouse γ2bκ (MOPC 195) from Litton Bionetics (Kensington, MD).

cDNA Clone Isolation and Characterization. A cDNA library was constructed using the pcD vector system described by Okayama and Berg (27), from poly(A)$^+$ RNA isolated from the human monocyte line U937, by a method similar to that of Chirgwin et al. (28). $\sim$10^5 clones were screened with a ^{32}P-labeled cDNA insert from pFcR11 encoding a mouse FcγR (Fig. 1, reference 25) as follows: competent *Escherichia coli* strain MC1061 cells were transformed with 1 ng of amplified pcD-U937 library DNA and plated on Luria broth plates containing ampicillin. For screening, colonies were transferred to circular Gene-Screen membranes, denatured, and neutralized according to protocols supplied by New England Nuclear (Boston, MA). Filters containing plasmid DNAs were prewashed in several changes of 3× SSC, 0.1% SDS at 60°C for 24 h. Prewashed filters were prehybridized in a solution containing 6× SSC, 1% SDS, 5× Denhardt's solution, 0.1% sodium pyrophosphate, 200 μg/ml denatured salmon sperm DNA, and 200 μg/ml denatured pcD plasmid DNA for 24 h at 37°C. Filters were hybridized under the same conditions containing the ^{32}P-labeled 5' 800-bp restriction fragment of the mouse β2 cDNA (pFcR11; Fig. 1) at a concentration of $\sim$5 ng/ml. After hybridization, filters were washed twice in 6× SSC at room temperature, followed by two washes in 2× SSC, 1% SDS at 42°C, and were exposed on Kodak XAR-Xomat film.

Three U937-pcD cDNA clones that crosshybridized to the mouse cDNA were purified and restriction maps were determined. Restriction fragments of one of these clones, designated 16.2, were subcloned into the m13mp10 and m13mp18 vectors and sequenced by the dideoxy method. Plasmid sequencing was performed using triple cesium-banded DNA and a modified procedure described by Hattori and Sakaki (29).

Transfection of Mouse Ltk⁻ Cells. Mouse Ltk⁻ cells to be transfected with human FcγRII clones were cultured in DME containing 2 mM L-glutamine, 10% FCS, 10 U/ml penicillin, and 10 μg/ml streptomycin. Confluent cells were trypsinized $\sim$16–18 h before transfection and were seeded at a density of 1–2 × 10^4 cells/well in eight-chambered slides (Miles Scientific Div., Naperville, IL). Cells were washed once with DME containing 0.025 M Tris, pH 7.4 (Tris-DME), immediately before transfection. The transfection solution contained 0.2 ml Tris-DME, 5 μg/ml plasmid DNA, and 400 μg/ml DEAE-dextran (Pharmacia Fine Chemicals, Piscataway, NJ). Cells were incubated with the transfection solution for 4 h, washed once with Tris-DME and incubated for 60 h at 37°C, 5% CO$_2$ in culture medium. In some experiments, cells were incubated for 3 h in DME containing 2% FCS and 100 μM chloroquine and washed before the 60-h incubation in culture medium. Mock transfections were performed exactly as described above, except plasmid DNA encoding human FcγRII was omitted from the transfection solution.

Immunofluorescent Staining of Transfected Mouse Ltk⁻ Cells. Growth medium was removed and transfected cells were washed with HBSS containing 10% FCS and 10 mM Hepes. All staining was performed on ice to prevent endocytosis of receptors. Human IgG was isolated from serum by ammonium sulfate precipitation, DEAE-cellulose chro-

matography, and gel filtration on AcA 44, and was heat aggregated as follows: IgG (1.7 mg/ml) was heated at 55°C for 20 min and centrifuged at 2,000 g for 10 min to remove large aggregates. The supernatant was used at a concentration of 50–100 μg/ml to assess expression of surface receptors for IgG by transfected cells. Human myeloma IgA (Cappell Laboratories, Malvern, PA) containing both monomeric and dimeric IgA was used as an isotype control. The second-stage reagents in the procedure were FITC-labeled F(ab')₂ fragments of goat anti–human IgG and IgA (Tago Inc., Burlingame, CA) or goat anti-mouse IgM (Zymed Laboratories, San Francisco, CA).

Transiently transfected cells were also examined for ability to bind anti-FcγR mAbs. Culture supernatants of hybridomas producing IV.3 (anti-FcγRII), 32 (anti-FcγRI), and 3G8 (anti-FcγRlo) were centrifuged at 12,000 rpm for 10 min before staining. Leu-11b was used at 10 μg/ml. Second-stage reagent was FITC-labeled F(ab')₂ fragments of goat anti–mouse IgG and IgM (Jackson Immunoresearch Laboratories, Avondale, PA).

All staining reagents were diluted in HBSS + 10% FCS + 10 mM Hepes buffer. Stained cells were mounted in a solution containing 2% N-propyl-gallate in 90% glycerol/10% PBS, pH 8.45.

RNA and DNA Blot Analysis.   Human placental genomic DNA was isolated as described (30). For RNA blot analysis, 4 μg/lane of poly(A)⁺ RNA was subjected to electrophoresis in a formaldehyde/1% agarose gel. The RNA was transferred to a Gene-Screen Plus membrane, baked at 80°C for 2 h, and prehybridized in 50% formamide, 1.0 M NaCl, 5× Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA for 6 h at 42°C. For genomic DNA blot analysis, genomic DNA was digested to completion with the indicated restriction endonuclease and 5 μg of each digest was electrophoresed on a 1% agarose gel. DNA was transferred to Gene Screen Plus, dried, and prehybridized as described above. Both filters were hybridized to a ³²P-labeled Xho I fragment encoding the entire human FcγRII cDNA in a solution containing 50% formamide, 1 M NaCl, 1% SDS, 1× Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA at 42°C. Filters were washed twice in 2× SSC, 1% SDS at 65°C for 30 min, then washed in 0.2× SSC, 0.1% SDS for 30 min at 30°C, and exposed for 18–100 h on Kodak XAR film with an intensifying screen at 90°C.

Generation of Antipeptide Antibodies by In Vitro Immunization.   Peptides were synthesized on an Applied Biosystems, Inc., (Foster City, CA) 430A peptide synthesizer, cleaved with hydrogen fluoride, and purified by preparative HPLC on a Waters Delta Prep system. Peptide identity was confirmed by amino acid analysis. Single cell suspensions from naive BALB/c mouse spleens were immunized in vitro (Vaux, D. J. T., and I. Mellman, unpublished observations). Briefly, spleen cells were resuspended at 2 × 10⁷ cells/ml in medium consisting of αMEM with 1 mM sodium pyruvate, 2 mM glutamine, 50 μM 2-ME, and 20% heat-inactivated FCS. Lyophilized peptides were resuspended in an equal volume of the above medium, which had been conditioned by mixed mouse thymocytes and sterilized by filtration, Antigen and cells were mixed and cultured at 37°C, 5% CO₂ in 75-cm² flasks for 5 d (18 μg/ml antigen). Nonadherent cells were collected, erythrocytes removed, and the remaining spleen cells fused with the SP-2 myeloma at spleen/myeloma ratio of 2:1 using 47% PEG 1500 (J. T. Baker Chemical Co., Phillipsburg, NJ) with 7.5% DMSO. The fused cells were plated in medium containing hypoxanthine and azaserine on a feeder layer of BALB/c mouse peritoneal exudate cells. Wells were fed on days 2, 4, and 6, and screening commenced on day 7.

Positive wells were identified by solid-phase ELISA. U937 cell lysates (10⁷ cells/ml in PBS containing 1% NP-40, 2 mM EDTA, and 0.22 U/ml aprotinin) were centrifuged at 1,800 g for 10 min to remove nuclei and were incubated overnight at 4°C in Immunolon plates at 30 μl/well. Wells were washed twice in PBS and blocked with PBS containing 8% goat serum for 2 h at 4°C. Candidate mAb supernatants were incubated in the coated wells for 1 h at 4°C and the plates were washed five times with PBS before a second PBS/8% goat serum blocking step. The second-stage reagent was goat anti–mouse IgG/IgM conjugated to alkaline phosphatase (Zymed Laboratories), 1:500 in PBS/8% goat serum. After a 1-h incubation, plates were washed five times with PBS, once with 50 mM sodium glycinate, pH 9.8, and developed with a Nitroblue tetrazolium/5-bromo-4-
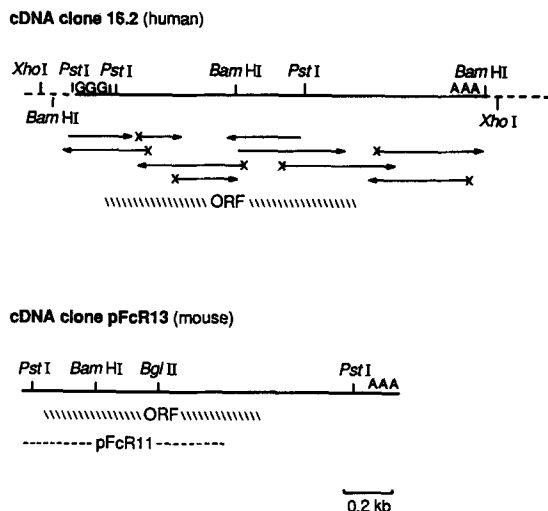
cDNA clone 16.2 (human)

cDNA clone pFcR13 (mouse)

FIGURE 1. Restriction map of the human FcγRII. cDNA (16.2) isolated from a U937-pcD library with indicated restriction sites (*top*), and the mouse homologue (FcγRβ2) of the human cDNA (*bottom*). An 800-bp fragment at the 5' end of the mouse FcγRβ2 cDNA (indicated by pFcR11) was used for screening the U937 library. Restriction fragments of the human cDNA were subcloned into m13mp10 and m13mp18, and sequenced according to the strategy indicated below the map by the arrowed bars. In several cases (✕), synthetic oligonucleotides complementary to FcγR sequence were used as primers. The open reading frames (*ORF*) of both mouse and human sequences are indicated by slanted bars located below each map. pcD vector sequence at the 5' and 3' ends of the human cDNA is shown as broken lines.

chloro-3-indolyl phosphate substrate at pH 9.8. Positive wells were expanded and the cells cloned twice in soft agar. Binding in the first screen was confirmed by indirect immunofluorescence and FACS analysis using live U937 cells. All mAbs were of the IgM subclass and showed no reactivity above background on HeLa or HepG2 human cell lines.

Preparation of Polyclonal Antipeptide Antisera. Polyclonal antipeptide antisera were raised in rabbits using a popliteal lymph node immunization protocol (31). Aqueous solutions of unconjugated peptides (2 mg/ml) were emulsified in an equal volume of CFA and a total of 200 μg of peptide was injected into the popliteal lymph nodes of unprimed rabbits. At 4 wk rabbits were boosted subcutaneously with 200 μg of peptide in IFA. 1 wk later an intravenous boost of 200 μg unconjugated peptide was given and blood was collected 10 d after the final boost.

Fluorescence Analysis of U937 and Daudi Cells. Both cell lines were maintained in RPMI 1640 supplemented with 10% FCS, L-glutamine, penicillin-streptomycin, and 50 μM 2-ME (Daudi only). Cells from logarithmically growing cultures were washed twice in HBSS containing 10% FCS and 10 mM Hepes buffer, and resuspended at 10^6 cells/ml in HBSS/FCS/Hepes before staining. All staining was performed on ice in glass tubes using 10^5 cells for each sample. Supernatant-containing IV.3 was used at a final dilution of 1:80 in HBSS/FCS/Hepes. The isotype control for IV.3 was MOPC 195 (γ2bκ) at 10 μg/ml. All washes were done with HBSS/FCS/Hepes. Second-stage reagents were as described above. Cells were analyzed using a FACS II or FACS IV (Becton Dickinson & Co., Mountain View, CA).

Results

Human FcγR cDNA Clones Homologous to a Mouse Fc[γ2b/γ1]R cDNA. The human monocyte cell line U937 expresses at least two of the classes of human FcγR (FcγRI and FcγRII; reference 16). We constructed a cDNA library from U937 poly(A)$^+$ RNA in the mammalian cell expression vector pcD (27). $\sim 10^5$ clones were screened using a ^{32}P-labeled 800-bp restriction fragment from the 5' end of a mouse FcγR cDNA clone (Fig. 1, reference 25) as a probe under conditions of reduced stringency. Three independent cDNA clones hybridizing to the probe were identified (16.1, 16.2, 17.1). All of the clones had cDNA inserts of ~ 1.6 kbp that exhibited identical restriction map patterns (Fig. 1). The complete DNA sequence of one of these clones (16.2) was determined (Fig. 2),

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          10                        34                  49                  64                  79                  94
GGGGGGGAC AGTGCTGGG ATG ACT ATG GAG ACC CAA ATG TCT CAG AAT GTA TGT CCC AGA AAC CTG TGG CTG CTT CAA CCA TTG ACA GTT TTG CTG CTG CTG
                    MET Thr MET Glu Thr Gln MET Ser Gln Asn Val Cys Pro Arg Asn Leu Trp Leu Leu Gln Pro Leu Thr Val Leu Leu Leu Leu
          +1
     109                       139                 154                 169                 184                 199
GCT TCT GCA GAC AGT CAA GCT GCA GCT CCC CCA AAG GCT GTG CTG AAA CTT GAG CCC CCG TGG ATC AAC GTG CTC CAG GAG GAC TCT GTG ACT CTG ACA
Ala Ser Ala Asp Ser Gln Ala Ala Ala Pro Pro Lys Ala Val Leu Lys Leu Glu Pro Pro Trp Ile Asn Val Leu Gln Glu Asp Ser Val Thr Leu Thr
       214   229                 244                 259                 274                 289
TGC CAG GGG GCT CGC CCT GAG AGC GAC TCC ATT CAG TGG TTC CAC AAT GGG AAT CTC ATT CCC ACC CAC ACG CAG CCC AGC TAC AGG TTC AAG GCC
Cys Gln Gly Ala Arg Ser Pro Glu Ser Asp Ser Ile Gln Trp Phe His Asn Gly Asn Leu Ile Pro Thr His His Thr Gln Pro Ser Tyr Arg Phe Lys Ala
     304                 319                 334                 349                 364                 379                 394
AAC AAC AAT GAC AGC GGG GAG TAC ACG TGC CAG ACT GGC CAG ACC CAG CTC AGC GAC CCT GTG CAT CTG GAT GTG CTT TCC CAA TGG CTG CTC CAG
Asn Asn Asn Asp Ser Gly Glu Tyr Thr Cys Gln Thr Gly Gln Thr Gln Leu Ser Asp Pro Val His Leu Asp Val Leu Ser Glu Trp Leu Val Leu Gln
     409                 424                 439                 454            469                 484                 499
ACC CCT CAC CTG GAG TTC CAG GAG GGA GAA ACC ATC ATG CTG AGG TGC CAC AGC TGG AGG GAC AAG CCT CTG GTC AAG GTC ACA TTC TTC CAG AAT GGA
Thr Pro His Leu Glu Phe Gln Glu Gly Glu Thr Ile MET Leu Arg Cys His Ser Trp Arg Asp Lys Pro Leu Val Lys Val Thr Phe Phe Gln Asn Gly
     514                 529                 544                 559                 574                 589
AAA TCC CAG AAA TTC TCC CGT TTG GAT CCC ACC TTC TCC ATC CCA CAA GCA AAC CAC AGT CAC AGT GGT GAT TAC CAC TGC ACA GGA AAC ATA GGC TAC
Lys Ser Gln Lys Phe Ser Arg Leu Asp Pro Thr Phe Ser Ile Pro Gln Ala Asn His Ser His Ser Gly Asp Tyr His Cys Thr Gly Asn Ile Gly Tyr
     619                 634            649                 664            679                 694
ACG CTG TTC TCA TCC AAG CCT GTG ACC ATC ACT GTC CAA GTG CCC CAA ATG GGC AGC TCT TCA CCA ATG GGG ATC ATT GTG GCT GTG ATC GCG ACT
Thr Leu Phe Ser Ser Lys Pro Val Thr Ile Thr Val Gln Val Pro Gln MET Gly Ser Ser Pro Met Gly Ile Ile Val Ala Val Ile Ala Thr
     709                 724                 739                 754            769                 784
GCT GTA GCC ATT GTT GTT GCC GTA GTG GCC TTG ATC TAC TGC AGG AAA AAG CGG ATT TCA GCC AAT TCC ACT GAT CCT GTG AAG GCT GCC CAA TTT
Ala Val Ala Ile Val Val Ala Val Val Ala Leu Ile Tyr Cys Arg Lys Lys Arg Ile Ser Ala Asn Ser Thr Asp Pro Val Lys Ala Ala Gln Phe
     799                 814                 829                 844                 859                 874                 889
GAG CCA CCT GGA CGT CAA ATG ATT GCC ATC AGA AAG AGA CAA CTT GAA GAA AAC CTT GAA GAA ACC AAT AAT GAC TAT GAA ACA GCT GAC GGC GGC TAC ATG ACT CTG AAC
Glu Pro Pro Gly Arg Gln MET Ile Ala Ile Arg Lys Arg Gln Leu Glu Glu Asn Leu Glu Glu Thr Asn Asn Asp Tyr Glu Thr Ala Asp Gly Gly Tyr MET Thr Leu Asn
     904                 919                 934                 949                 964                 983                 993
CCC AGG GCA CCT ACT GAC GAT AAA AAC AAC ATC TAC CTG ACT CTT CCT CCT CCA AAC GAC CAT GTC AAC AGT AAT AAC TAA AGAGTAACGT TATGCCATGT
Pro Arg Ala Pro Thr Asp Asp Lys Asn Asn Ile Tyr Leu Thr Leu Pro Pro Pro Asn Asp His Val Asn Ser Asn Asn
     1003       1013       1023       1033       1043       1053       1063       1073       1083       1093       1103       1113
GGTCATACTC TCAGCTTGCG TATGGATGCA AAAAGAGGG GAATTGTTAA AGGAAAATTT AAATGGACAC CTGAGCAAAC AAAACCACCT GGCCCTTAGA AATAGCTTTA
     1123       1133       1143       1153       1163       1173       1183       1193       1203       1213       1223       1233
ACTTTGCTTA AACTACAAAC ACAAGCAAAA CTTCACGGGG TCATACTACA TACAAGCATA TACAACCATA AGCAAAACTT AACTTGGATC ATTTCTGGTA AATGCTTATG TTAGAAATAA GACAACCCCA
     1243       1253       1263       1273       1283       1293       1303       1313       1323       1333       1343       1353
GCCAATCACA AGCAGCCCTAC TAACATATAA TTAGGTGACT TTAGGGACTTTC TAAGAAGATA CCTACCCCCA AAAACACAATT ATGTAATTGA AAACCAACCG ATTGCCTTTA TTTTGCTTCC
     1363       1373       1383       1393       1403
ACATTTCCC AATAAATACT TGCCTGTGAC ATTTTGCCAC TGGAACACTA (A)n
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FIGURE 2. The nucleotide sequence of the human cDNA clone, 16.2, and deduced amino acid sequence of FcγR. The NH₂-terminal signal sequence is ~30 amino acids in length and is followed by an extracellular domain of ~180 amino acids. +1, above the nucleotide sequence, indicates the start of the extracellular domain, by analogy with the mouse sequence. Three potential N-linked glycosylation sites are boxed. Two glycosylation sites that have not been conserved between the human and mouse sequences are indicated by broken boxes. Cysteine residues that have potential to form intrachain disulfide bonds are underlined. Peptides used for in vitro immunization are shown with broken overbars. The decapeptide is labeled (1) and the octapeptide is labeled (2). The putative transmembrane domain has been indicated with an overbar beginning at nucleotide 656. The COOH-terminal cytoplasmic domain consists of 76 amino acids, terminates at nucleotide residue 973, and contains one potential site for N-linked glycosylation. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00644.

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hFcR   (8)     VLKLEPPWINVLQEDSVTLTCQGARSPESD---SIQW--FHNGNLIPTHTQ
               I     I   I    III I  I  I I I   I     I       I
hCSFR  (22)    VIEPSVPELVVKPGATVTLRCVGNGSVEWDGPPSPHWTLYSDGSSSILSTN


hFcR   (54)    PSYRFKANNNDSGEYTCQTGQTSLSDP----VYLT-VLSEWLVLQTPHLEF
               I     I I I    I      I   I         I  I II    I
hCSFR  (72)    NA-TFQ----NTGTYRCTEPGDPLGGSAAIHLYVKDPARPWNVLAQEVVVF


hFcR   (100)   QEGETIMLRCHSWKDKPLVKVTRRQNGKSQKFSR-----LDP--TFSIPQA
               I I   I   I           I           I     I  I I  I
hCSFR  (117)   EDQDA-LLPCL-LTDPVLEAGVSLVRVRGRPLMRHTNYSFSPWHGFTIHRA


hFcR   (144)   NHSHSGDYHCTGNIGYTLFSSKPVTITVQV
               I II I    I      I      II
hCSFR  (165)   KFIQSQDYQCSALMGGRKVMSISIRLKVQK
```

FIGURE 3. Homology between human FcγR (residues 8–173) and human CSF-1 receptor (34; residues 22–195). Overall homology is 42/165 amino acids, or ~25%.

and a putative amino acid sequence was deduced. The cDNA clone encodes a protein of 324 amino acids (36 kD) with a structure very similar to that described for the mouse Fc[γ2b/γ1]R. The NH₂-terminus contains a hydrophobic signal sequence of up to 30 amino acids, with three possible AUG initiation codons. This region is followed by a predicted extracellular domain of ~180 amino acids, which, as described for the mouse protein (25, 26), contains two tandem homology units exhibiting significant sequence homology to members of the Ig gene superfamily. The repeating units are defined by pairs of cysteine residues that are surrounded by sequences characteristic of Ig variable regions (32). However, like the mouse FcγR and neural cell adhesion molecule (N-CAM) sequences (25), the number of amino acids between each pair of cysteine residues is too small to account for the typical variable region folding pattern.

A comparison of the translated human sequence (Fig. 2) to the National Biomedical Research Foundation protein sequence data base using the program IFIND (Intelligenetics, Inc., Mountain View, CA) revealed a number of significant homologies to Ig-like molecules, including the epithelial cell receptor for polymeric IgA/IgM (poly-IgR; reference 33), the receptor for platelet-derived growth factor, neural cell adhesion molecule (N-CAM), and several proteins encoded by various mouse MHC and human HLA loci.

Using the ALIGN program (34) in conjunction with an Ig family sequence data base (kindly made available by A. F. Williams, MRC Laboratory, Oxford, United Kingdom), a particularly strong homology was found between both Ig-like domains of the human FcγR and amino acids 22–195 of the human CSF-1 receptor/c-fms proto-oncogene (35), a plasma membrane protein also typical of cells of the mononuclear phagocyte lineage. The best match yielded a highly significant alignment score of 7.32 SD units (32, 34; Fig. 3).

The extracellular domain is followed by a long and markedly hydrophobic stretch that probably represents the receptor's membrane-spanning segment. By analogy to the more extensively studied mouse FcγR, we presume that this transmembrane domain is about 29 amino acids long (overlined, Fig. 2). We note that this region is preceded by 13 amino acids (nucleotides 617–655) that are not markedly hydrophilic.

The extracellular domain contains two potential sites for N-linked glycosylation (boxed, Fig. 2) that are conserved between the mouse and human FcγR se-

quences. Two additional sites identified in the mouse FcγR (24–26) are not conserved in the human protein as a consequence of three amino acid substitutions (broken boxes; Fig. 2). The presumed cytoplasmic domain contains one potential site for N-linked glycosylation (nucleotides 764–772, Fig. 2), although it is unlikely to be used. The cytoplasmic domain is unusually long (76 amino acids), and no significant homology of this region to cytoplasmic domains of other receptors was detected.

Human FcγR cDNA Clones Represent a Novel FcγR mRNA Structure. Three distinct mouse Fc[γ2b/γ1]R cDNA clones have been described (26), designated α, β1, and β2. These cDNAs share identical or nearly (95%) identical sequences in their extracellular domains, but show marked differences elsewhere. Both FcγRβ cDNAs are identical except for a 46 amino acid in-frame insertion in the predicted cytoplasmic tail of FcγRβ1. In contrast, the FcγRα cDNA contains a unique set of sequences comprising the 5'- and 3'-untranslated regions, signal sequence, transmembrane domain, and cytoplasmic domain.

We compared the deduced amino acid sequence of the human FcγR with the available mouse FcγR sequences (Fig. 4, *a* and *b*). Overall homology between the human and mouse extracellular domains is 61% at the amino acid sequence level (75% nucleotide sequence homology). However, the signal sequence of human FcγR shares substantial homology (60%) only with mouse FcγRα; only 23% homology exists between the human and FcγRβ signal sequences (Fig. 4*b*). Conversely, the putative transmembrane domain shares ~50% amino acid homology with the corresponding region of the mouse FcγRβ cDNA clones, but has virtually no similarity to FcγRα. The 76 amino acid cytoplasmic domain of the human protein is unrelated to any of the mouse cDNAs. The amino acid sequence comparisons summarized in Table I show that the sequence of the human FcγR cDNA consists of segments closely related to both the murine α and β cDNAs, as well as a unique cytoplasmic domain.

The Human FcγR cDNA Clone Hybridizes to Two Distinct mRNA Species in Human FcγR-expressing Cell Lines. Poly(A)$^+$ RNA from several human cell lines was subjected to electrophoresis on a formaldehyde-agarose gel and examined by RNA blot hybridization with the human FcγR cDNA insert as a probe. The cDNA insert was isolated from pcD as an Xho I fragment (27). Fig. 5*a* shows that, in addition to a 1.6-kb mRNA corresponding in size to clone 16.2, a 2.5-kb species was also detected in the U937 and HL60 cell lines. The same pattern was detected in RNA isolated from human peripheral blood mononuclear cells (data not shown). No corresponding 2.5-kb mRNA was detected by crosshybridization to P388D1 (mouse macrophage) RNA, although the 1.6-kb species is seen (25, 26). RNA from an FcγR$^-$ B-lymphoblastoid cell line (RPMI8866, reference 36) and a human T cell hybridoma (166A2, reference 37) did not hybridize to the FcγR probe. The 1.6-kb species and a trace of the 2.5-kb mRNA are also expressed by the FcγR-bearing B-lymphoblastoid cell line Daudi (Fig. 5*a*; reference 36). The relative intensities of the 1.6-kb bands in U937 and Daudi RNAs suggest that this mRNA is more abundant in Daudi cells than in U937.

The FcγR cDNA was also used as a probe of restriction digests of human placental DNA. The probe hybridizes to several both weak and intense bands
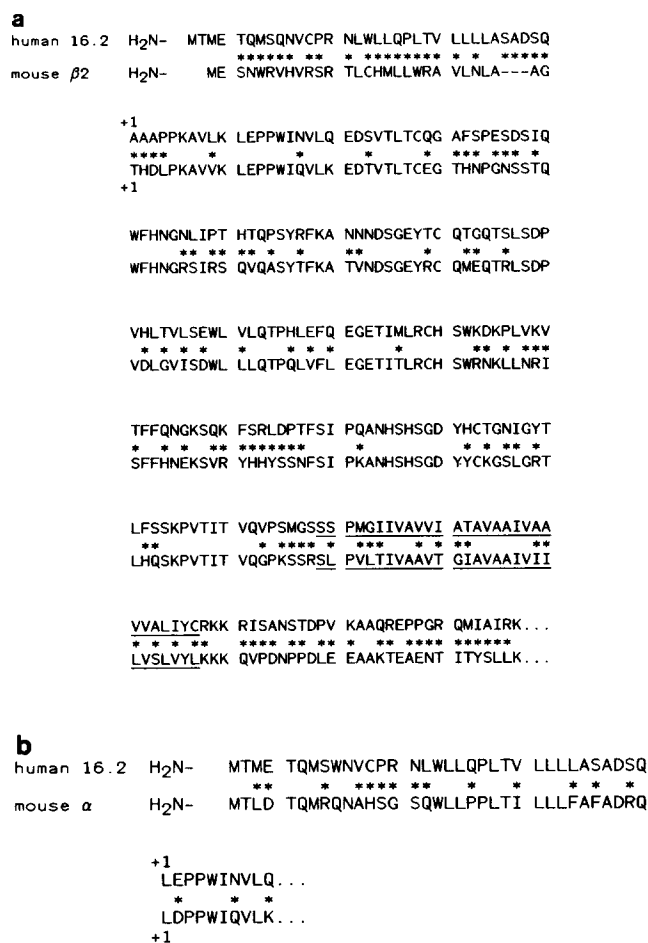
a

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human 16.2  H2N-  MTME  TQMSQNVCPR  NLWLLQPLTV  LLLLASADSQ
                        ******  **  *  ********  *  *  *****
mouse  β2    H2N-    ME  SNWRVHVRSR  TLCHMLLWRA  VLNLA---AG


            +1
            AAAPPKAVLK  LEPPWINVLQ  EDSVTLTCQG  AFSPESDSIQ
            ****    *        *      *  ***  ***  *
            THDLPKAVVK  LEPPWIQVLK  EDTVTLTCEG  THNPGNSSTQ
            +1


            WFHNGNLIPT  HTQPSYRFKA  NNNDSGEYTC  QTGQTSLSDP
            **  **  **  *    *        **       *  **   *
            WFHNGRSIRS  QVQASYTFKA  TVNDSGEYRC  QMEQTRLSDP


            VHLTVLSEWL  VLQTPHLEFQ  EGETIMLRCH  SWKDKPLVKV
            *  *  *  *    *    *  *  *       *    **  *  ***
            VDLGVISDWL  LLQTPQLVFL  EGETITLRCH  SWRNKLLNRI


            TFFQNGKSQK  FSRLDPTFSI  PQANHSHSGD  YHCTGNIGYT
            *   *   *  **  *******  *           *  *  **  *
            SFFHNEKSVR  YHHYSSNFSI  PKANHSHSGD  YYCKGSLGRT


            LFSSKPVTIT  VQVPSMGSSS  PMGIIVAVVI  ATAVAAIVAA
            **      *  ****  *   ***     *  *  **        **
            LHQSKPVTIT  VQGPKSSRSL  PVLTIVAAVT  GIAVAAIVII


            VVALIYCRKK  RISANSTDPV  KAAQREPPGR  QMIAIRK...
            *  *  *  **  ****  **  **  *   **  ****  ******
            LVSLVYLKKK  QVPDNPPDLE  EAAKTEAENT  ITYSLLK...
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b

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human 16.2  H2N-  MTME  TQMSWNVCPR  NLWLLQPLTV  LLLLASADSQ
                        **      *   ****  **   *    *    *  *  *
mouse  α     H2N-  MTLD  TQMRQNAHSG  SQWLLPPLTI  LLLFAFADRQ


            +1
            LEPPWINVLQ...
            *     *    *
            LDPPWIQVLK...
            +1
```

FIGURE 4. Homology of human and mouse Fcγ receptor proteins. (a) The predicted amino acid sequences of the human FcγRII and mouse FcγRβ2 have been aligned. The start of the extracellular domains of the human and mouse proteins is indicated (+1) above the first amino acid in the sequence of this domain. The transmembrane domains have been underlined. Asterisks indicate differences between the aligned sequences. The homology between the putative human and mouse β2 proteins begins in the extracellular domain and extends through the transmembrane domain. The cytoplasmic portion of this human receptor exhibits little homology to that of mouse FcγRβ2. (b) The predicted signal sequences of the human FcγRII and mouse FcγRα are homologous. Little homology (<15%) is observed between the transmembrane or cytoplasmic regions of the putative human FcγR and mouse FcγRα proteins. The beginning of the mouse extracellular domain and corresponding region of the mature human FcγR sequence are indicated (+1). Homology between the human FcγR and mouse FcγRα amino acid sequences in the extracellular domain is comparable to that observed between the human FcγR and mouse FcγRβ proteins.

TABLE I

Amino Acid Sequence Homology of Human and Mouse FcγR

Domain of protein	S	E	TM	C
		%		
16.2:mouse-FcγRα	60	ND	—	—
16.2:mouse-FcγRβ1	23	61	52	—
16.2:mouse-FcγRβ2	23	61	52	—

Homology searches were conducted for sets of protein sequences (human vs. mouse α, human vs. mouse β1, and human vs. mouse β2) and results were tabulated. Slashes indicate where there was no significant homology (<15%) between structural domains of the putative human and mouse proteins. Domains of FcγR have been abbreviated as follows: S, signal sequence; E, extracellular; TM, transmembrane; C, cytoplasmic domain.
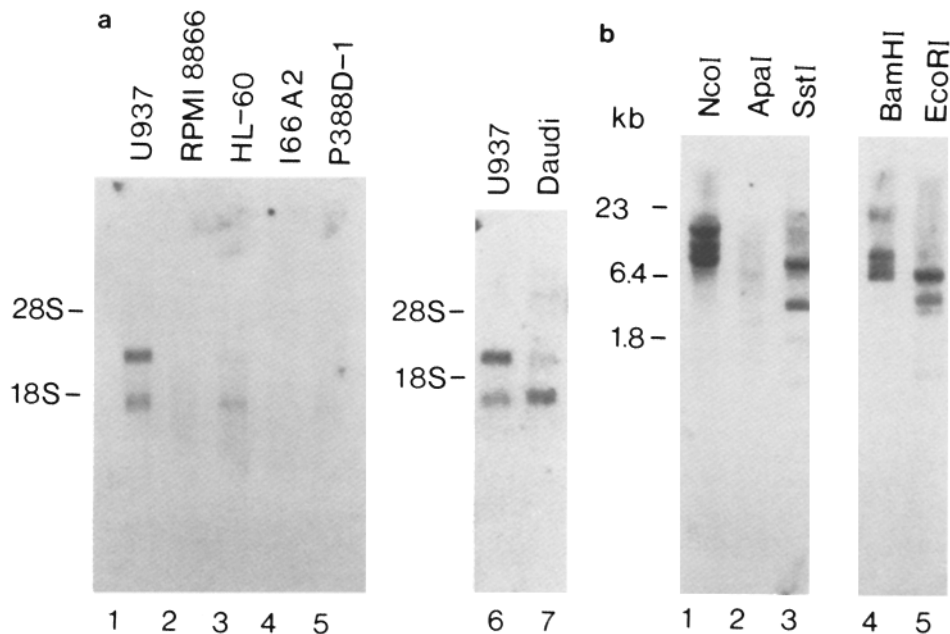
FIGURE 5.　RNA and genomic DNA blot analyses of the human FcγR genes homologous to cDNA clone 16.2. (a) 5 μg of poly(A) selected RNA from human lines U937 (*1* and *6*), RPMI8866 (*2*), HL-60 (*3*), 166A2 (*4*), Daudi (*7*), and the mouse macrophage line, P-388D1 (*5*) were electrophoresed in a formaldehyde-agarose gel, transferred to Gene-Screen Plus, and probed with a nick-translated Xho I fragment encoding the entire human FcγRII cDNA. Positions of 28S and 18S ribosomal RNAs are indicated by arrows. (b) 5-μg aliquots of human placental DNA were digested with Nco I (*1*), Apa I (*2*), Sst I (*3*), Bam HI (*4*) and Eco RI (*5*), electrophoresed in a 1% agarose gel, transferred to Gene Screen Plus, and probed with an Xho I fragment representing the human FcγR cDNA.

(Fig. 5*b*) in each restriction digest, ranging in size from <2 kb to >10 kb; in none of the digests examined was only a single band observed.

The Human FcγR cDNA Clone Encodes FcγRII.　Mouse Ltk⁻ cells transiently transfected with FcγR clone 16.2 were tested for expression of both ligand-binding activity and antigenic determinants recognized by anti–human FcγR mAbs. As shown in Fig. 6, cells transfected with the cDNA clone express functional Fc receptors that bind heat-aggregated human IgG (Fig. 6*a*). These receptors exhibit specificity for IgG because binding of human IgA to the transfected cells was not detected (Fig. 6*b*). Mouse Ltk⁻ cells subjected to a mock transfection procedure were unable to bind either IgG or IgA (Fig. 6,*c* and *d*). Identical results were obtained with cDNA clones 16.1 and 17.1 (data not shown).

Cells transfected with the FcγR cDNA clone were next tested for reactivity with several anti–human FcγR mAbs. No staining of transfected cells was observed with the anti–FcγRlo mAbs 3G8 (19) and Leu-11b (22) (data not shown), or the anti–FcγRI antibody 32 (Fig. 6*e*, reference 18). However, the anti–FcγRII antibody IV.3 (16) specifically stained Ltk⁻ cells transfected with the FcγR cDNA clone (Fig. 6, *f* and *g*). All mAbs were obtained from culture supernatants and were thus devoid of additional mouse IgG present in ascites
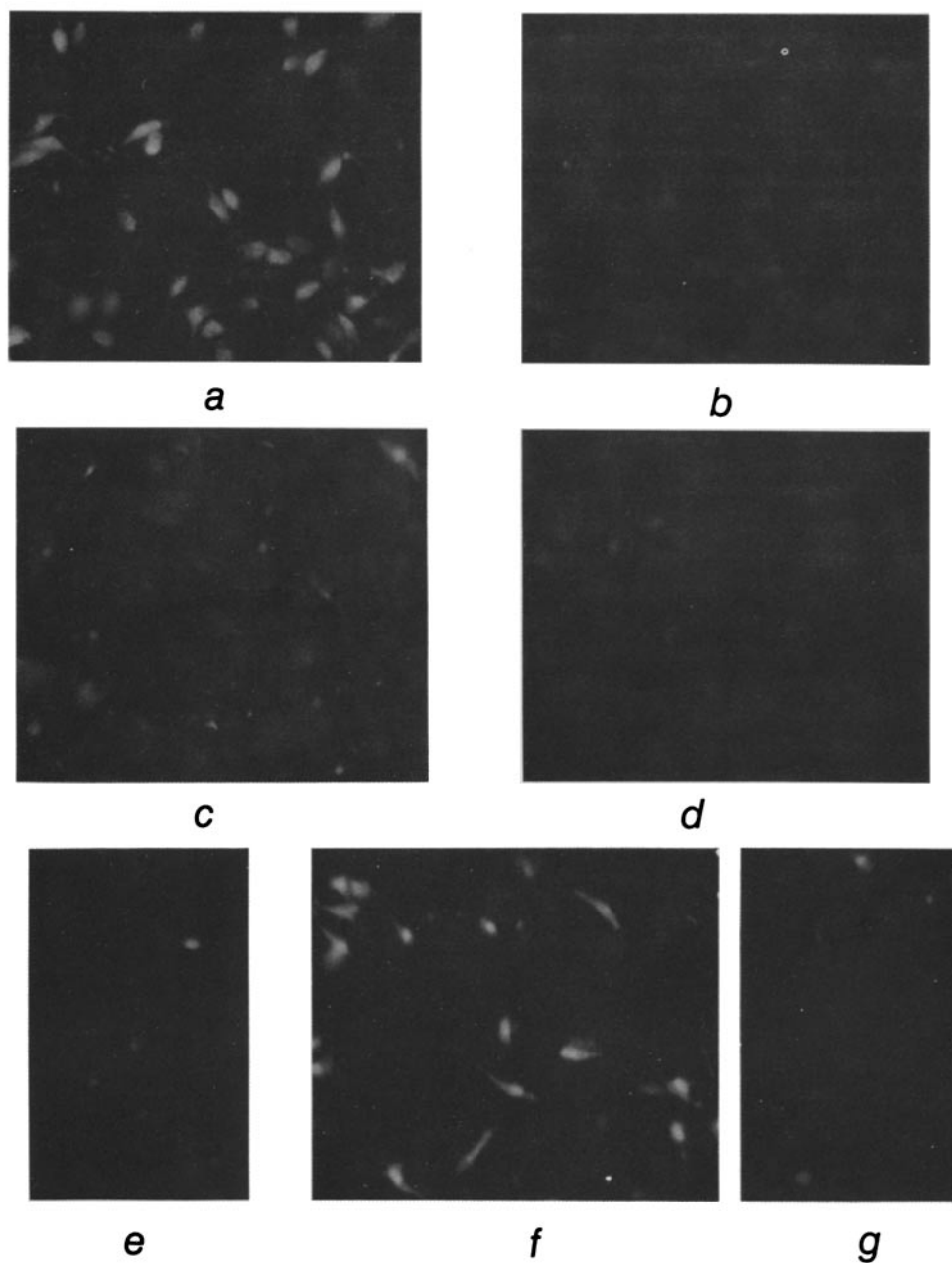
FIGURE 6. Immunofluorescence staining of mouse Ltk⁻ cells transfected with human cDNA clone 16.2. Mouse Ltk⁻ cells transiently transfected with 16.2-pcD plasmid DNA were assessed for their ability to bind human IgG (a) and anti-FcγRII antibody IV.3 (f). Ltk⁻ cells transfected with clone 16.2 did not bind human myeloma IgA (b), nor were they stained by anti-FcγRI antibody 32 (e). Only background fluorescence was observed in mock transfectants stained with either IgG/anti-IgG (c), IgA/anti-IgA (d), or with mAb IV.3 (g).
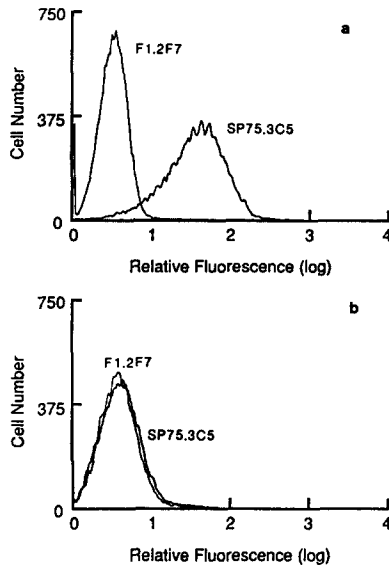
FIGURE 7. Flow cytometric analysis of U937 and Daudi cells stained with antipeptide antibodies. (a) U937 cells stained with the anti-FcγRII peptide antibody SP75.3C5 and a control antibody, F1.2F7. (b) Daudi cells stained as in a.

preparations. A control experiment with IgG2b (MOPC 195) of irrelevant specificity gave negative results (data not shown).

The FcγR Encoded by Clone 16.2 is Expressed by a Human Monocyte Cell Line. Of the three cDNA clones encoding mouse Fc[γ2b/γ1]R, only the FcγRβ cDNAs have been expressed by transfection (26; Koch, T., et al., unpublished data). Furthermore, it has thus far proved difficult to detect proteins corresponding to either FcγRα or FcγRβ1 in mouse macrophage and lymphocyte cell lines (Koch, T., and I Mellman, unpublished data). To establish that clone 16.2 encodes an FcγR actually expressed by human cells, we tested human monocyte (U937) and FcγR⁺ B-lymphoblastoid (Daudi) cell lines for expression of FcγR related to this cDNA clone using antipeptide antibodies.

Two synthetic peptides were prepared, an 8-mer and 10-mer (broken overlines, Fig. 2). These peptides corresponded to regions of the FcγRII sequence likely to be present on the B β-strands stabilized by the intrachain disulfides in Ig-like domains (32, 38), and were selected with the aid of the program PEP (Intelligenetics, Inc.) The unconjugated peptides were used first for production of mouse mAbs using a modified procedure for in vitro immunization (Vaux, D. J. T., and I. Mellman, manuscript in preparation). Since this procedure yields only antibodies of the IgM class, the resulting antipeptide antibodies were not likely to bind nonspecifically to FcγR. Supernatants harvested after the initial 4-d immunization and from several IgM-secreting hybridoma cell lines were used to stain receptor-positive and receptor-negative cells by indirect immunofluorescence. As shown by flow cytometry in Fig. 7, specific staining of U937 cells was observed with any of several mAbs to the octapeptide (SP75.3C5). No staining was observed using an irrelevant monoclonal IgM specific for a viral glycoprotein (F1.2F7; Fig. 7a). The anti–FcγR peptide antibodies failed to stain HeLa and HepG2 cells, both FcγR-negative human cell lines (data not shown). Interestingly, the antipeptide antibodies also failed to stain Daudi cells appreciably (Fig. 7b),
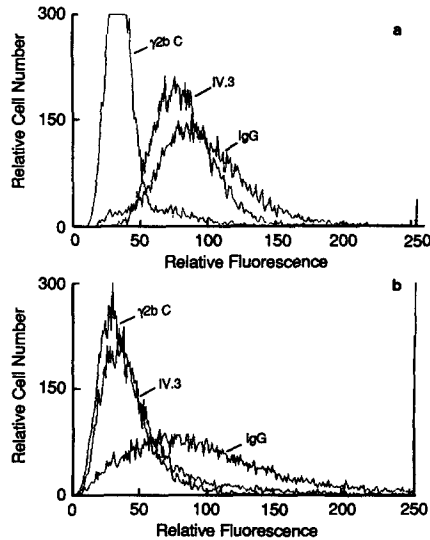
FIGURE 8. Flow cytometric analysis of U937 and Daudi cells stained with aggregated IgG and an anti-FcγRII mAb. (a) U937; (b) Daudi. Fluorescence is on a linear scale.

although these cells express a 1.6-kb mRNA homologous to the FcγRII probe (Fig. 5a). Similar results were obtained using a conventional rabbit polyclonal antiserum produced by injection of the unconjugated octapeptide directly into the popliteal lymph nodes. The antipeptide antibody SP75.3C5 also stained Ltk⁻ and Chinese hamster ovary cells transfected with the FcγRII cDNA (data not shown). These results demonstrate that cDNA clone 16.2 encodes a functional FcγR expressed on the surface of the U937 monocyte cell line.

U937 and Daudi cells were also analyzed for binding to aggregated IgG and anti-FcγRII antibody IV.3. Fig. 8 shows that U937 was stained with both IgG and IV.3. Daudi cells stained to a comparable extent with IgG, but only slight staining with IV.3 was detected, suggesting that the FcγRII expressed by these two cell lines are related (16; Fig. 5a), but not identical (Figs. 7 and 8).

Discussion

We have cloned and determined the molecular structure of a human monocyte Fc receptor for IgG. Transfection of the cloned cDNA in mouse Ltk⁻ cells results in expression of a surface receptor, specific for human IgG, which is recognized by the anti-FcγRII mAb IV.3, but not by a number of other anti-FcγR mAbs. Moreover, antibodies against a peptide derived from the cloned sequence stain the parent U937 cell line. We therefore conclude that clone 16.2 encodes the human FcγRII class of FcγR expressed on monocytes. The predicted size of the FcγRII encoded by 16.2 (36 kD with two potential sites for N-linked glycosylation) is also consistent with the observed size of FcγRII on human monocytes and B cells: 40 kD (16). The cloned FcγRII has ~75% DNA sequence homology and 61% protein sequence homology in the extracellular domain with the mouse Fc receptor for IgG2b and IgG1. We therefore believe that FcγRII is the human homologue of mouse Fc[γ2b/γ1]R. This relationship has been suggested previously (14, 16) based on several properties of the human and mouse receptors: their similar sizes, their preference for binding aggregated IgG, and the ability

of the human FcγRII to bind mouse IgG1 and IgG2b, but not mouse IgG2a. Our results demonstrate this conclusion at the molecular level.

The lack of reactivity of the cloned, expressed FcγRII with mAbs specific for the FcγRI and FcγRlo classes of human FcγR clearly distinguishes FcγRII from the latter two FcγR. However, it is still possible that this molecule may be structurally related to FcγRI and FcγRlo. The human FcγRII cDNA probe does detect a mRNA species in U937 that is larger (2.5 kb; Fig. 5) than that represented by 16.2; no corresponding 2.5-kb mRNA has been reported in mouse cells expressing FcγR. The possibility that this larger mRNA could represent one of the other two classes of human FcγR is under investigation.

The human B cell line Daudi has a 40-kD Fc receptor for IgG (16) and appears to have more of the 1.6-kb mRNA homologous to the FcγRII probe than does U937 (Fig. 5). However, Daudi cells were not stained by either the anti–FcγRII antibody IV.3 or anti–FcγRII peptide antibodies (Figs. 7 and 8). These results are consistent with those reported by Looney et al. (16), who showed that IV.3 could precipitate FcγRII from U937 but not from Daudi cells. Our data suggest that the IV.3 and antipeptide antibodies recognize determinants on FcγRII which are either masked or not present when this receptor is expressed in Daudi cells. Alternatively, the FcγRII-like molecule on Daudi cells may be encoded by an FcγRII gene that is closely related to but distinct from that contained in clone 16.2. A related situation may also exist for the mouse Fc[γ2b/γ1]R. A rat anti-FcγR mAb (6B7C) detects this antigen only on activated B lymphocytes and some tumor cell lines, but not on macrophages and resting lymphocytes (Pure, E., M. D. Widmer, J. B. Lum, I. Mellman, and J. C. Unkeless, manuscript submitted for publication).

The possibility of multiple gene segments related to FcγRII is also suggested by genomic blot analysis (Fig. 5). With any of the restriction enzymes used, multiple large bands hybridizing to the FcγRII probe are detected. Because humans are not an inbred species, it is difficult to interpret these observations definitively. However, the data suggest that either the genomic FcγR gene(s) may exhibit restriction site polymorphism, the FcγR genomic gene(s) contain several large intervening sequences, or that the FcγR cDNA is homologous to a small multigene family comprising several members.

Our expression and genomic blot data suggest two possible explanations for a reported polymorphism in human monocyte FcγRII (39). Monocytes of a large proportion of Caucasians support proliferation of human T cells mediated by the anti-T3 mAb Leu-4 (IgG1), while those of a majority of Asians do so only poorly. Anderson et al. (39) have demonstrated an association of distinct isoelectric focusing patterns of FcγRII with these "responder/nonresponder" phenotypes, and suggested that human FcγRII is encoded by a single gene with two allelic variants. This explanation may be correct if there are several large introns in the genomic FcγRII gene, or if there is restriction site polymorphism associated with the phenotypic polymorphism. Alternatively, the observed polymorphism could be explained by a difference in control of cell-type specificity of expression of individual members of an FcγRII gene family. In this light, we note again that FcγRII expressed on a B cell line is antigenically distinct from monocyte FcγRII (Figs. 7 and 8), and supports Leu-4-mediated T cell proliferation only

poorly (16). We thus speculate that nonresponder individuals might express an FcγRII on their monocytes that is restricted to other cell types in responders.

The FcγRII cDNA clone has a signal sequence related to the mouse FcγRα cDNA, a putative transmembrane region homologous to the mouse FcγRβ cDNA, and an extracellular domain closely related to both types of mouse FcγR (25, 26). Thus, it is apparent that in human FcγR-expressing cells, a single FcγR protein may contain sequences related to both the α and β cDNAs of the mouse. These findings indicate that the α- and β-specific gene segments are not uniquely associated with a particular gene segment encoding the extracellular domain of FcγR.

The FcγRII represented by clone 16.2 contains a unique, rather long cytoplasmic domain, which is unrelated to the mouse FcγR cytoplasmic domains or to those of other known receptor molecules. This observed heterogeneity of FcγR cytoplasmic domains remains unexplained. When such comparisons are made, it is unusual for interspecies homologues of cell surface molecules to diverge to a greater extent in their cytoplasmic domains than in their extracellular domains. The lack of similarity among FcγR cytoplasmic domains is particularly interesting in this case, since one can presume that the homologous FcγR should perform very similar functions in mouse and human macrophages, respectively. Either these functions do not require a particular primary structure for the cytoplasmic domain, or there remain several other members of an FcγR gene family yet to be isolated. It is possible that further study of ligand-dependent effector functions of FcγR such as endocytosis, antibody-dependent cellular cytotoxicity, superoxide production, and formation of bioactive lipids will clarify this issue and suggest functional roles for the various cytoplasmic domains of FcγR.

Like its murine counterparts, human FcγRII exhibits homology to proteins encoded by members of the Ig gene superfamily (poly-IgA/IgM receptor, MHC class II antigen, N-CAM). We have also found a significant homology to a portion of the human CSF-1 receptor. The extracellular domains of both mouse and human FcγR have a characteristic structural feature: there are only 42–44 amino acid residues between cysteines, while Ig, poly-IgR, and MHC antigens have at least 80 residues between cysteines that form disulfide linkages. The truncated domains appear to lack at least two of the β-strands typically found in Ig-like domains (31). In this respect, FcγR is closely related to N-CAM; the CSF-1 receptor also exhibits truncated Ig-like domains and is likewise related to human and mouse FcγR.

Anti-FcγRII peptide antibodies generated as described here may be very useful reagents for the study of FcγRII expression. They are the first amino acid sequence–specific antibodies produced against any human FcγR; that they are of the IgM class renders further manipulation (i.e., isolation of Fab fragments) unecessary. The FcγRII cDNA clone and its complementary antipeptide antibodies should make possible a thorough study of the expression of FcγRII on human hematopoietic cells and of its relationship to other classes of human FcγR.

Summary

We have cloned and expressed a cDNA encoding a human receptor for IgG (FcγR) from the monocyte cell line U937. The deduced structure is a 35-kD transmembrane protein with homology to the mouse Fc[γ2b/γ1] receptor amino acid sequence of ~60% in the extracellular domain. The signal sequence is homologous to the mouse FcγRα cDNA clone, while the transmembrane domain shares homology with mouse FcγRβ cDNAs. The cytoplasmic domain is apparently unique. The extracellular domain shows significant homology to proteins of the Ig gene superfamily, including the human c-fms protooncogene/CSF-1 receptor. Mouse Ltk⁻ cells transfected with the human FcγR cDNA express a cell-surface receptor that selectively binds human IgG and is recognized by the anti-FcγRII mAb IV.3. Antibodies against peptides derived from the human FcγR sequence specifically stain U937 cells, but not an FcγRII-bearing B-lymphoblastoid cell line (Daudi). These results identify the human FcγRII as the homologue of mouse Fc[γ2b/γ1]R, and provide evidence for heterogeneity of FcγRII expressed on monocytes and B cells.

Note added in proof: Dr. Brian Seed (Massachusetts General Hospital) has also isolated cDNAs encoding human Fcγ RII (CDw32). The sequence of his cDNA clone is identical to 16.2 in the protein-coding region, but differs at six positions in the 3'-untranslated region.

References

1. Mellman, I. S., H. Plutner, R. H. Steinman, J. C. Unkeless, and Z. A. Cohn. 1983. Internalization and degradation of macrophage Fc receptors during receptor-mediated phagocytosis. *J. Cell Biol.* 96:887.
2. Kurlander, R. J., D. M. Ellison, and J. Hall. 1984. The blockade of Fc receptor-mediated clearance of immune complexes *in vivo* by a monoclonal antibody (2.4G2) directed against Fc receptors on murine leukocytes. *J. Immunol.* 133:855.
3. Mellman, I., and H. Plutner. 1984. Internalization and degradation of macrophage Fc receptors bound to polyvalent immune complexes. *J. Cell Biol.* 98:1170.
4. Nathan, C., and Z. Cohn. 1980. Role of oxygen-dependent mechanisms in antibody-induced lysis of tumor cells by activated macrophages. *J. Exp. Med.* 152:198.
5. Kipps, T. J., P. Parham, J. Punt, and L. A. Herzenberg. 1985. Importance of immunoglobulin isotype in human antibody-dependent cell-mediated cytotoxicity directed by murine monoclonal antibodies. *J. Exp. Med.* 161:1.
6. Rouzer, C. A., W. A. Scott, J. Kempe, and Z. A. Cohn. 1980. Prostaglandin synthesis by macrophages requires a specific receptor-ligand interaction. *Proc. Natl. Acad. Sci. USA.* 77:4279.
7. Fridman, W. H., C. Rabourdin-Combe, C. Neauport-Sautes, and R. M. Gisler. 1981. Characterization and function of T cell Fcγ receptor. *Immunol. Rev.* 56:51.
8. Bich Thuy, L. T., and J. P. Revillard. 1982. Selective suppression of human B

lymphocyte differentiation into IgG-producing cells by soluble Fc receptors. *J. Immunol.* 129:150.

9. Cohen, L., S. Sharp, and A. Kulczycki. 1983. Human monocytes, B lymphocytes, and non-B lymphocytes each have structurally unique Fc receptors. *J. Immunol.* 131:378.

10. Anderson, C. L., and R. J. Looney. 1986. Human leukocyte IgG Fc receptors. *Immunol. Today.* 7:264.

11. Dickler, H. B. 1977. Lymphocyte receptors for immunoglobulin. *Adv. Immunol.* 24:167.

12. Anderson, C. L., and G. N. Abraham. 1980. Characterization of the Fc receptor for IgG on a human macrophage cell line, U937. *J. Immunol.* 125:2735.

13. Kulczycki, A. 1984. Human neutrophils and eosinophils have structurally distinct Fc receptors. *J. Immunol.* 133:849.

14. Jones, D. H., R. J. Looney, and C. L. Anderson. 1985. Two distinct classes of IgG Fc receptors on a human monocyte line (U937) defined by differences in binding of murine IgG subclasses at low ionic strength. *J. Immunol.* 135:3348.

15. Lukeck, M. D., Z. Steplewski, F. Baglia, M. H. Klein, K. J. Dorrington, and H. Koprowski. 1985. The interaction of murine IgG subclass proteins with human monocyte Fc receptors. *J. Immunol.* 135:1299.

16. Looney, R. J., G. N. Abraham, and C. L. Anderson. 1986. Human monocytes and U937 cells bear two distinct Fc receptors for IgG. *J. Immunol.* 136:1641.

17. Clement, L. T., A. B. Tilden, and N. E. Dunlap. 1985. Analysis of the monocyte Fc receptors and antibody-mediated cellular interactions required for the induction of T cell proliferation by anti-T3 antibodies. *J. Immunol.* 135:165.

18. Anderson, C. L., P. M. Guyre, J. C. Whitin, D. H. Ryan, R. J. Looney, and M. W. Fanger. 1986. Monoclonal antibodies to Fc receptors for IgG on human mononuclear phagocytes: antibody characterization and induction of superoxide production in a monocyte cell line. *J. Biol. Chem.* 261:12856.

19. Fleit, H. B., S. D. Wright, and J. C. Unkeless. 1982. Human neutrophil Fc receptor distribution and structure. *Proc. Natl. Acad. Sci. USA.* 79:3275.

20. Perussia, B., O. Acuto, C. Terhost, J. Faust, R. Lazarus, V. Fanning, and G. Trinchieri. 1983. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. II. Studies of B73.1 antibody-antigen interaction on the lymphocyte membrane. *J. Immunol.* 130:2142.

21. Vaughn, M., M. Taylor, and T. Mohanakumar. 1985. Characterization of human IgG Fc receptors. *J. Immunol.* 135:165.

22. Perussia, B., G. Trinchieri, A. Jackson, H. L. Warner, J. Faust, H. Rumpold, D. Kraft, and L. L. Lanier. 1984. The Fc receptor for IgG on human natural killer cells: phenotypic, functional, and comparative studies with monoclonal antibodies. *J. Immunol.* 133:180.

23. Unkeless, J. C., H. Fleit, and I. S. Mellman. 1981. Structural aspects and heterogeneity of immunoglobulin Fc receptor. *Adv. Immunol.* 31:247.

24. Green, S. A., H. Plutner, and I. Mellman. 1985. Biosynthesis and intracellular transport of the mouse macrophage Fc receptor. *J. Biol. Chem.* 260:9867.

25. Lewis, V. A., T. Koch, H. Plutner, and I. Mellman. 1986. A complementary DNA clone for a macrophage-lymphocyte Fc receptor. *Nature (Lond.).* 324:372.

26. Ravetch, J. V., A. D. Luster, R. Weinshank, J. Kochan, A. Pavlovec, D. A. Portnoy, J. Humes, Y.-C. E. Pan, and J. C. Unkeless. 1986. Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. *Science (Wash. DC).* 234:718.

27. Okayama, H., and P. Berg. 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. *Mol. Cell. Biol.* 3:280.

28. Chirgwin, J. M., A. E. Pryzbyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*. 18:5294.

29. Hattori, M., and Y. Sakaki. 1986. Dideoxy sequencing method using denatured plasmid templates. *Anal. Biochem.* 152:232.

30. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 280–281.

31. Reggio, H., D. Bainton, E. Harms, E. Coudrier, and D. Louvard. 1984. Antibodies against lysosomal membranes reveal a 100,000 mol wt protein that cross-reacts with purified H^+K^+-ATPase. *J. Cell Biol.* 99:1511.

32. Barclay, A. N., P. Johnson, G. W. McCaughan, and A. F. Williams. 1988. Immunoglobulin-related structures associated with vertebrate cell surfaces. *In* The T-Cell Receptors. T. Mak, editor, Plenum Press, New York. In press.

33. Mostov, K. E., M. Friedlander, and G. Blobel. 1984. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature (Lond.).* 308:37.

34. Dayhoff, M. O., W. C. Barker, and L. T. Hunt. 1983. Establishing homologies in protein sequences. *Methods Enzymol.* 91:524.

35. Coussens, L., C. Van Beveren, D. Smith, E. Chen, R. L. Mitchell, C. M. Isacke, I. M. Verma, and A. Ullrich. 1986. Structural alteration of viral homologue of receptor protooncogene fms at carboxyl terminus. *Nature (Lond.).* 320:277.

36. Speigelberg, H. L. 1981. Lymphocytes bearing Fc receptors for IgE. *Immunol. Rev.* 56:199.

37. Huff, T. F., and K. Ishizaka. 1984. Formation of human IgE-binding factors by human T-cell hybridomas. *Proc. Natl. Acad. Sci. USA.* 81:1514.

38. Amzel, L. M., and R. J. Poljak. 1979. Three-dimensional structure of immunoglobulins. *Annu. Rev. Biochem.* 48:961.

39. Anderson, C. L., D. H. Ryan, R. J. Looney, and P. C. Leary. 1987. Structural polymorphism of the human monocyte 40 kilodalton Fc receptor for IgG. *J. Immunol.* 138:2254.