STRUCTURE AND EXPRESSION OF HUMAN IgG FcRII(CD32)

Functional Heterogeneity Is Encoded by the
Alternatively Spliced Products of Multiple Genes

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Cell surface receptors for the Fc domain of IgG are found on most cells of the hematopoietic lineage and are defined by their ability to bind IgG-antigen complexes. This binding couples the humoral and cellular immune response and is a component in an organism's capacity to clear foreign antigens. In the human, these receptors have been divided into three classes based on differences in apparent molecular mass, affinity for IgG, cellular distribution and reactivity with mAbs (reviewed in reference 1). FcRI is a high affinity Fc receptor, binding monomeric IgG, and is expressed on monocytic cells. FcRs II and III are low affinity receptors, binding immune complexes of IgG, and are expressed on both myeloid and lymphoid cells. The broad distribution of FcRII is mirrored in the array of cellular responses attributed to these receptors. Crosslinking of FcRII on monocytes and granulocytes by immune complexes results in effector responses such as phagocytosis, antibody-dependent cellular cytotoxicity (ADCC),[1] and the release of mediators of inflammation (2). Engagement of these receptors on lymphocytes is suggested to be involved in the regulation of lymphocyte differentiation and antibody production (3).

The structural basis for this functional heterogeneity in response to a common ligand was first elucidated for the murine low affinity IgG Fc receptors, murine FcRII. Two genes, α and β, encode this class of receptors and display nearly identical ligand binding domains coupled to divergent membrane spanning and intracytoplasmic domains (4–6). α is expressed on macrophages and NK cells (4, 7, 8), while β is expressed on both myeloid and lymphoid cells. Alternative splicing of a cytoplasmic exon of the β gene is tissue specific, generating further diversity in the intracytoplasmic domains of these receptors. The implication of those data are that functional heterogeneity results from the divergent membrane spanning and intracytoplasmic domains.

Analysis of the human homologues of the low affinity IgG Fc receptors lends additional support to this model. In man, two classes of low affinity receptors have been defined by virtue of their apparent molecular mass, reactivity with mAbs and cel-

[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; hu, human; mu, murine; PCR, polymerase chain reaction.

lular distribution (9). FcRII(CD32) is expressed on most myeloid and lymphoid cells, while FCRIII(CD16) expression is restricted, appearing on NK cells, macrophages, and neutrophils. Recent studies have demonstrated that FcRIII(CD16) exists in two alternative membrane anchored forms, which result from tissue specific expression of two genes differing by single nucleotide substitutions (10). A transmembrane anchored form of FcRIII(CD16) is expressed on NK cells and macrophages, demonstrating homology in its transmembrane and intracytoplasmic domains to murine FcR α. The neutrophil molecule, in contrast, is anchored by a phosphatidylinositol linkage, yet is nearly identical in sequence to the NK molecule in its extracellular domains. These differentially anchored molecules respond to the same ligand with distinctly different responses. ADCC is mediated by the FcRIII(CD16) molecule on NK cells, yet the neutrophil receptor is unable to mediate this response independently (11-13). In contrast, the structural diversity of human FcRII(CD32) has been largely underestimated. Previous reports have described the isolation of one complete cDNA clone (referred to here as FcRIIa) and a partial clone derived from a second gene (14-16). Paradoxically, evolutionary conservation of human homologues for the murine β molecules appeared to be absent based on those reports. In this study we describe the characterization of multiple cDNA clones for FcRII(CD32) that arise from three distinct genes. One group of clones correspond to transcripts derived from a human homologue of a β gene, here referred to as FcγRIIb, demonstrating conservation in sequence, alternative splicing pattern and tissue distribution to its murine counterpart. Another clone is derived from a gene referred to as FcγRIIa, which represents a chimera of α and β sequences, while a third FcγRII(CD32) gene and its corresponding transcript, referred to as FcγRIIa', has structural features of both FcγRIIa and IIb. The FcγRIIa and IIb molecules can be transfected into heterologous cells and express low affinity IgG binding molecules. Both the IIa and IIb gene products are recognized by mAbs of the CD32 cluster.

Materials and Methods

cDNA Isolation and Characterization. Human genomic FcγRII DNA fragments were isolated by screening a human genomic library constructed by partial Mbo I digestion of human placenta DNA and ligated into the λ phage vector CH28. 500,000 plaques were screened with a nick-translated 1.3-kb Pst I fragment containing the murine β_1 FcγR cDNA insert (4) at reduced stringency (25% formamide, 5× SSC, 7 mM Tris, pH 7.5, 10% dextran sulfate, and 25 μg/ml sheared salmon sperm DNA at 42°C). Two positive clones were identified, plaque purified, and further characterized by restriction mapping. A 1.3-kb Bgl II fragment was identified by its hybridization to the murine β_1 cDNA and further analyzed by DNA sequence analysis. Two exons were found on that fragment that had 60% amino acid homology to the second extracellular and transmembrane domains of the murine β_1 FcγR. Thus, by virtue of its homologous sequence and genomic organization, this 1.3-kb Bgl II fragment was determined to encode a human FcγRII gene fragment. This genomic fragment was used to isolate FcγRIIa from a λgt 10 cDNA library (kindly provided by Drs. Xue-Dong Fan and B. Bloom, Albert Einstein College of Medicine, New York, NY) made from poly(A)$^+$ RNA isolated from the human monocyte-like U937 cell line (17). FcγRIIb1 and IIb2 were isolated from a λgt 10 cDNA library (kindly provided by Drs. A. Corbi and T. Springer, Center for Blood Research, Boston, MA) made from poly(A)$^+$ RNA isolated from PMA-treated HL-60 cells (18). FcγRIIb3 and the truncated FcγRIIb clone were isolated from a λgt 10 cDNA library (kindly provided by Drs. J. DiSanto and N. Flomenberg, Sloan-Kettering Institute, New York, NY) made from Daudi poly(A)$^+$ RNA. FcγRIIb cDNAs were isolated using a radiolabeled overlapping oligonucleotide probe based on the previously published

signal sequence of a partial IIb clone (16). FcγRIIa' was isolated from a genomic cosmid library by screening with mixed FcγRIIa and FcγIIb probes. Its corresponding transcript was isolated by reverse transcription followed by PCR amplification of neutrophil, monocyte, and lymphocyte RNA with oligonucleotide probes specific to the IIb 5' untranslated sequences and IIa cytoplasmic sequences. All cDNA library screening was done as previously described (4). For DNA sequencing cDNA inserts were subcloned into pUC-9 and both strands were sequenced by the chain termination method (19).

Characterization of Cellular RNA. RNA isolation, poly(A) selection, and Northern blot analysis were performed as previously described (4). In brief, RNA (2 μg poly(A)$^+$ RNA per sample in Fig. 6, 10 μg total RNA from primary cells) was fractionated on a 2.2 M formaldehyde–1% agarose gel and transferred to nitrocellulose. The filter was hybridized at high stringency (50% formamide, 42°C) to probes consisting of overlapping oligonucleotides synthesized to the divergent signal sequences of each gene. Specific activities of 10^8 cpm/μg were obtained by labeling with α-[^{32}P]dCTP and *Escherichia coli* polymerase (Klenow fragment). Rehybridization of filters involved the removal of previously hybridized probe by heating at 65°C for 1 h in 50 mM Tris, pH 8.0, 2 mM EDTA, 1× Denhardt's, and 0.5% sodium pyrophosphate.

For analysis of cellular RNA by the polymerase chain reaction (PCR), 10 μg of total RNA was reverse transcribed into cDNA and amplified as previously described (10) with the following modifications. Oligonucleotides were made as described (10) to sequences in the 5' untranslated region or signal sequence and cytoplasmic domain or 3' untranslated region that allowed specific hybridization at an annealing temperature of 50°C. Aliquots of each amplification reaction were fractionated on agarose gels and subjected to Southern blot analysis as previously described (4). Overlapping oligonucleotide probes made to distinct domains of FcγRII sequences were labeled and hybridized as above to determine the structure of these PCR amplified cDNAs.

Cell Culture. Cell lines HL-60, U937, THP-1, and MOLT-4 were from American Type Culture Collection (Rockville, MD). K562 was provided by Dr. R. Knowles; AW Ramos, Daudi, and Raji were provided by Dr. J. Lee; IM-9 was provided by Dr. O. Rosen; and Ltk$^-$ cells were from Dr. N. Flomenberg all of Sloan-Kettering Institute (New York, New York). K562, HL-60, U937, IM-9, and Ltk$^-$ cells were maintained in α-modified MEM supplemented with 10% FCS (Gibco Laboratories, Grand Island, NY) heat inactivated at 56°C for 30 min, 100 U/ml penicillin, and 100 μg/ml streptomycin. Daudi, Raji, AW Ramos, and MOLT-4 were maintained in RPMI 1640 supplemented with 25 mM Hepes, pH 7.4, FCS, and antibiotics as above. THP-1 was grown in the same RPMI with 5 × 10^{-5} M β-mercaptoethanol.

Primary cells including human monocytes, neutrophils, and NK cells were prepared as previously described (10). B lymphocytes were prepared from a single human spleen by separating nonadherent mononuclear cells by Ficoll centrifugation and adhering to plastic twice (45 min, 37°C). B lymphocytes were purified by negative selection using antiglobulin rosetting with a mixture of mAbs (CD16, CD56, CD3, CD14, CD5) to deplete T and NK cells. Populations were >95% purity by indirect immunofluorescence flow cytometry. Human term placenta was from a spontaneous vaginal delivery and immediately washed in PBS and frozen in liquid nitrogen.

Gene Transfer and Rosetting Assays. Coding sequences of all FcγRII cDNAs were cloned into the Sma I site of the eukaryotic expression vector pCEXV-3. Transient and stable transfection and rosetting were done as previously described (7). In brief, murine Ltk$^-$ cells were incubated with FcγRII/pCEXV-3 plasmid and DEAE-dextran for 16 h, washed, and allowed to grow for 2 d before rosetting. Transfectants were assayed for rosette formation with TNP haptenated SRBCs opsonized with anti-DNP murine IgG1 mAb (U-7-6; kindly provided by Dr. Zelig Eschar, Weizman Institute, Rehovot, Israel). Stable transfectants were generated by calcium phosphate co-transfection with neomycin resistance conferring plasmid pGCcos3neo and selection with Geneticin (G418; Gibco Laboratories) as previously described (7). Stable transfectants were selected by rosette formation with murine IgG1 opsonized SRBCs (above) or with murine anti-FcγRII mAbs followed by SRBC conjugated with goat anti-mouse IgG Abs.

mAbs and Indirect Immunofluorescence.   mAbs used in this study were IV.3 (20), KuFc79 (21), and those of the CD32 cluster (22) including CIKM5, 2A1, KB61, and 41H16. 5 × 10⁵ stable transfectant cells were incubated with 1:500 dilutions of ascites for 45 min at 4°C. Cells were then washed in PBS with 2% FCS and 0.05% sodium azide three times at 4°C. Affinity-purified, FITC-conjugated goat anti-mouse IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added to washed cells at 10 µg/ml and incubated at 4°C for 45 min. Cells were washed as above and fixed in PBS/1% formaldehyde. The samples were analyzed on a Becton Dickinson FACScan 1 cytofluorometer using a Consort 30 data analysis program.

Results

Isolation of cDNA Clones for FcRII(CD32).   The structural homology of human FcRII(CD32) proteins to their murine counterparts was apparent in their ligand binding specificity for both murine and human IgG, their tissue distribution and apparent molecular mass. This structural relationship was exploited in the isolation of human cDNA clones for this receptor class. DNA probes derived from the murine α and β cDNA sequences were used to screen human genomic libraries at reduced stringency, resulting in the isolation of genomic clones that, as shown by sequence analysis, demonstrated 60% sequence identity in the exons encoding the extracellular domains to their murine homologues (not shown). Probes derived from these exons were used to screen cDNA libraries constructed from RNA derived from hematopoietic cell lines that were positive for FcRII(CD32), as determined by their reactivity with mAbs specific for this receptor. The monocytic line U937, the promyelocytic line HL-60 (differentiated with PMA to macrophage-like cells) and the B lymphoid line Daudi were screened with these presumptive FcRII(CD32) probes and positive clones were characterized by DNA sequence analysis. A schematic representation of these clones is shown in Fig. 1. Three classes of clones were ob-

FcγR II Transcripts
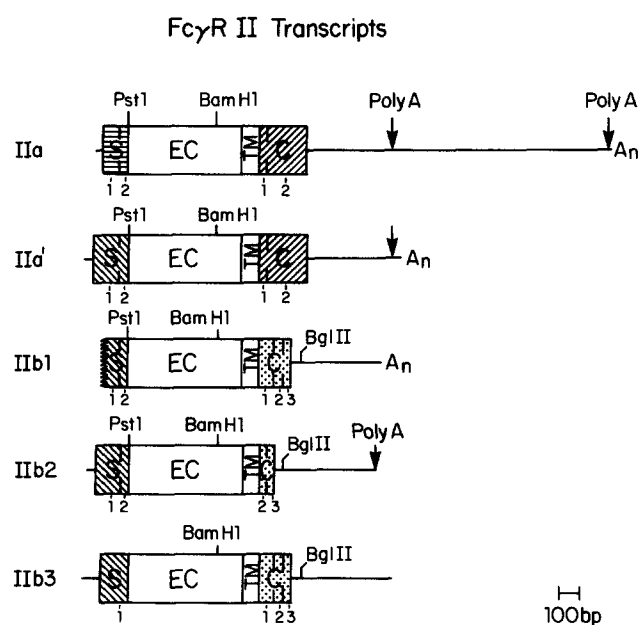
FIGURE 1. Structure of human FcγRII transcripts and partial restriction map of their respective cDNAs. Five cDNAs derived from three genes designated IIa, IIa', and IIb have been identified. FcγRIIb1-3 are alternatively spliced transcripts of the b gene. Untranslated sequences are indicated by a line and coding sequences by rectangles. The broken edge in FcγRIIb1 indicates an incomplete coding region. The signal sequences (S), extracellular domains (EC), transmembrane domain (TM), and cytoplasmic (C) regions are indicated. Regions of >90% nucleotide identity are indicated by common crosshatching, open areas, or stippling. Representative restriction sites are shown. Numbers below a box indicate the exon organization in alternatively spliced regions. Consensus polyadenylation sites are shown and poly(A) tails are indicated as Aₙ.

tained, designated IIa, IIa', and IIb. As will be described below, while these clones demonstrate nearly identical extracellular and transmembrane domains they differ considerably in the intracytoplasmic regions (IIa/a' vs. IIb) predicted by these sequences. In addition, three distinct IIb clones have been isolated that are the result of alternative RNA splicing of both NH2-terminal and COOH-terminal encoding exons.

Isolation and Sequence Analysis of Human FcRIIa cDNA. A U937 cDNA library was screened with the genomic FcγRII probe described above. Two positive phage were plaque purified and found to be identical by DNA sequence analysis. The complete nucleotide and predicted amino acid sequence of those FcγRIIa clones are shown in Fig. 2. An open reading frame of 951 nucleotides, beginning at position 8, predicts a mature FcγRIIa protein of 282 amino acids of molecular mass of 31,276 daltons


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            -35           -30                          -20
            M  A  M  E  T  Q  M  S  Q  N  V  C  P  R  N  L  W  L  L  Q  P  L  T
TTCTGGGATGGCTATGGAGACCCAAATGTCTCAGAATGTATGTCCCAGAAACCTGTGGCTGCTTCAACCATTGAC    75
         -10                              -1 +1                        10
         V  L  L  L  L  A  S  A  D  S  Q  A  A  A  P  P  K  A  V  L  K  L  E  P  P
AGTTTTGCTGCTGCTGGCTTCTGCAGACAGTCAAGCTGCAGCTCCCCCAAAGGCTGTGCTGAAACTTGAGCCCCC    150
                20                          30
W  I  N  V  L  Q  E  D  S  V  T  L  T  (C) Q  G  A  R  S  P  E  S  D  S  I
GTGGATCAACGTGCTCCAGGAGGACTCTGTGACTCTGACATGCCAGGGGGCTCGCAGCCCTGAGAGCGACTCCAT    225
      40                          50                          60
Q  W  F  H  N  G  N  L  I  P  T  H  T  Q  P  S  Y  R  F  K  A  N  N  [N  D]
TCAGTGGTTCCACAATGGGAATCTCATTCCCACCCACACGCAGCCCAGCTACAGGTTCAAGGCCAACAACAATGA    300
         [S] G  E  Y  T (C) Q  T  G  Q  T  S  L  S  D  P  V  H  L  T  V  L  S  E  W
CAGCGGGGAGTACACGTGCCAGACTGGCCAGACCAGCCTCAGCGACCCTGTGCATCTGACTGTGCTTTCCGAATG    375
               90                   100                       110
L  V  L  Q  T  P  H  L  E  F  Q  E  G  E  T  I  M  L  R (C) H  S  W  K  D
GCTGGTGCTCCAGACCCCTCACCTGGAGTTCCAGGAGGGAGAAACCATCATGCTGAGGTGCCACAGCTGGAAGGA    450
               120                         130
K  P  L  V  K  V  T  F  F  Q  N  G  K  S  Q  K  F  S  R  L  D  P  T  F  S
CAAGCCTCTGGTCAAGGTCACATTCTTCCAGAATGGAAAATCCCAGAAATTCTCCCGTTTGGATCCCACCTTCTC    525
      140                         150                         160
I  P  Q  A  [N  H  S] H  S  G  D  Y  H (C) T  G  N  I  G  Y  T  L  F  S  S
CATCCCACAAGCAAACCACAGTCACAGTGGTGATTACCACTGCACAGGAAACATAGGCTACACGCTGTTCTCATC    600
               170                       180
K  P  V  T  I  T  V  Q  V  P  S  M  G  S  S  S  P  M  G  I  I  V  A  V  V
CAAGCCTGTGACCATCACTGTCCAAGTGCCCAGCATGGGCAGCTCTTCACCAATGGGGATCATTGTGGCTGTGGT    675
         190                         200                       210
I  A  T  A  V  A  A  I  V  A  A  V  V  A  L  I  Y  C  R  K  K  R  I  S  A
CATTGCGACTGCTGTAGCAGCCATTGTTGCTGCTGTAGTGGCCTTGATCTACTGCAGGAAAAAGCGGATTTCAGC    750
               220                       230
N  S  T  D  P  V  K  A  A  Q  F  E  P  P  G  R  Q  M  I  A  I  R  K  R  Q
CAATTCCACTGATCCTGTGAAGGCTGCCCAATTTGAGCCACCTGGACGTCAAATGATTGCCATCAGAAAGAGACA    825
      240                         250                         260
L  E  E  T  N  N  D  Y  E  T  A  D  G  G  Y  M  T  L  N  P  R  A  P  T  D
ACTTGAAGAAACCAACAATGACTATGAAACAGCTGACGGCGGCTACATGACTCTGAACCCCAGGGCACCTACTGA    900
               270                       280
D  D  K  N  I  Y  L  T  L  P  P  N  D  H  V  N  S  N  N  *
CGATGATAAAAACATCTACCTGACTCTTCCTCCCAACGACCATGTCAACAGTAATAACTAAAGAGTAACGTTATG    975
CCATGTGGTCATACTCTCAGCTTGCTAGTGGATGACAAAAAGAGGGGAATTGTTAAAGGAAAATTTAAATGGAGA   1050
CTGGAAAAATCCTGAGCAAACAAAACCACCTGGCCCTTAGAAATAGCTTTAACTTTGCTTAAACTACAAACACAA   1125
GCAAAACTTCACGGGGTCATACTACATACAAGCATAAGCAAAACTTAACTTGGATCATTTCTGGTAAATGCTTAT   1200
GTTAGAAATAAGACAACCCCAGCCAATCACAAGCAGCCTACTAACATATAATTAGGTGACTAGGGACTTTCTAAG   1275
AAGATACCTACCCCCAAAAAACAATTATGTAATTGAAAACCAACCGATTGCCTTTATTTTGCTTCCACATTTTCC   1350
CAATAAATACTTGCCTGTGACATTTTGCCACTGGAACACTAAACTTCATGAATTGCGCCTCAGATTTTTGCTTTA   1425
ACATCTTTTTTTTTTTTTGACAGAGTCTCAATCTGTTACCCAGGCTGGAGTGCAGTGGTGCTATCTTGGCTCACT   1500
GCAAACCCGCCTCCCAGGTTTAAGCGATTCTCATGCCTCAGCCTCCCAGTAGCTGGGATTAGAGGCATGTGCATC   1575
ATACCCAGCTAATTTTTGTATTTTTTATTTTTTATTTTTAGTAGAGACAGGGTTTCGCAATGTTGGCCAGGCGAT   1650
CTCGAACTTCTGGCCTCTAGCGATCTGCCGCCTCGGCCTCCCAAAGTGCTGGGATGACCAGCATCAGCCCCAATG   1725
TCCAGCCTCTTTAACATCTTCTTTCCTATGCCCTCTCTGTGGATCCCTACTGCTGGTTTCTGCCTTCTCCATGCT   1800
GAGAACAAAATCACCTATTCACTGCTTATGCAGTCGGAAGCTCCAGAAGAACAAAGAGCCCAATTACCAGAACCA   1875
CATTAAGTCTCCATTGTTTTGCCTTGGGATTTGAGAAGAGAATTAGAGAGGTGAGGATCTGGTATTTCCTGGACT   1950
AAATTCCCCTTGGAAGACGAAGGGATGCTGCAGTTCCAAAAGAGAAGGACTCTTCCAGAGTCATCTACCTGAGTC   2025
CCAAAGCTCCCTGTCCTGAAAGCCACAGACAATATGGTCCCAAATGACTGACTGCACCTTCTGTGCCTCAGCCGT   2100
TCTTGACATCAAGAATCTTCTGTTCCACATCCACACAGCCAATACAATTAGTCAAACCACTGTTATTAACAGATG   2175
TAGCAACATGAGAAACGCTTATGTTACAGGTTACATGAGAGCAATCATGTAAGTCTATATGACTTCAGAAATGTT   2250
AAAATAGACTAACCTCTAACAACAAATTAAAAGTGATTGTTTCAAGGTGATGCAATTATTGATGACCTATTCTAT   2325
TTGTCTATAATGATCATATATTACCTTTGTAATAAAACATTATAATCAAAAAAAAAAAAAAAAAA            2389
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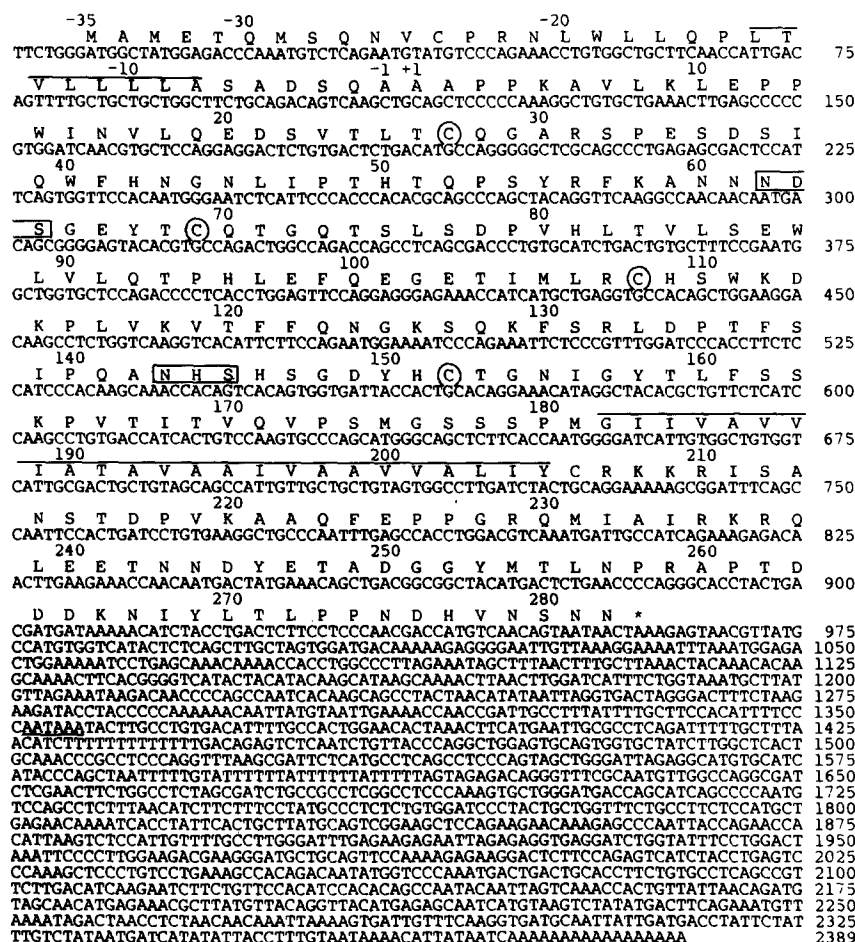
FIGURE 2. The translated sequence of FcγRIIa is presented in one letter code above the nucleotide sequence, which is numbered at the right. A 35 amino acid signal sequence is predicted and numbered -35 to -1 with an overlined hydrophobic core. Cysteine residues predicted to form disulfide bridges are circled and N-linked glycosylation sites are boxed. A hydrophobic stretch of 24 amino acids presumed to span the membrane is overlined. An asterisk denotes the stop codon and two consensus polyadenylation sites are underlined.

that is identical to that reported previously (14–16). The extracellular region is divided into two domains, each of which has homology to the Ig C2 set that centers around two highly conserved cysteine residues. Each extracellular domain retains one of the two sites for N-linked glycosylation that are found in the murine FcγRs to which this clone is homologous (discussed in detail below). A 28 amino acid hydrophobic transmembrane domain is followed by a 76 amino acid intracellular region. The 3' untranslated region of FcγRIIa contains two consensus polyadenylation signals at positions 1352 and 2356 (underlined in Fig. 2). The presence of a stretch of A residues that are not encoded in genomic DNA downstream of the second AATAAA indicates that this clone was derived from a mRNA that was polyadenylated just after this site.

Isolation and Analysis of FcγRIIb cDNAs. The isolation of FcγRIIb cDNAs was achieved by screening λgt10 cDNA libraries made from the B cell line Daudi and a macrophage-like cell line obtained by phorbol ester–induced differentiation of HL-60 cells. Multiple clones were isolated from each library and subjected to restriction mapping, which revealed four unique groups of cDNAs. Representatives of each group were purified and their cDNA inserts were subjected to DNA sequence analysis. These clones are shown schematically in Fig. 1; DNA sequences of three cDNAs with unique coding regions are shown in Fig. 3. A fourth incompletely processed cDNA is discussed below. The 1.5-kb FcγRIIb2 cDNA (Fig. 1) contains an open reading frame beginning at position 81 (Fig. 3) that predicts a protein of 247 amino acids (Fig. 4). A 44 amino acid signal sequence is predicted to be removed from this precursor generating a mature protein of 27,178 daltons. The extracellular domains of this protein and FcγRIIa are highly related, having 96% amino acid identity. This identity includes the four cysteines (circled in Fig. 4) that are predicted to form two disulfide-bridged Ig-like domains. FcγRIIb2 predicts three N-linked glycosylation sites in the extracellular region. The predicted transmembrane anchor contains a hydrophobic core of 23 amino acids followed by a basic stop transfer sequence arg-lys-lys-arg. A 44 amino acid intracellular region, largely unrelated to FcγRIIa, predicted by this cDNA is particularly rich in acidic residues (23%, Fig. 4). The FcγRIIb2 cDNA has a 3' untranslated region of 487 residues that ends one nucleotide after the poly(A) addition signal underlined in Fig. 3.

FcγRIIb1 is a cDNA sequence of 1,417 nucleotides that is incomplete in that it lacks sequences predicting the NH2-terminal 19 amino acids of signal sequence found in FcγRIIb2. However, genomic DNA sequence analysis indicates that FcγRIIb1 begins in the middle of an exon that encodes signal sequences in all other FcγRIIb cDNAs (Qiu, W. Q., and J. V. Ravetch, unpublished observations). It is likely, therefore, that this clone was truncated during construction of the cDNA library. FcγRIIb1 is identical to IIb2 from positions 139 through 840 (Fig. 3), which corresponds to sequences encoding the extracellular, transmembrane, and the beginning of the intracellular region. At that position a 57 nucleotide insertion is found in the FcγRIIb1 cDNA after which the two sequences are once again identical through the end of the coding regions. This insertion maintains the open reading frame and is known from genomic cloning to correspond precisely to an exon. These observations, together with the fact that a 74% amino acid identity to murine FcγR β1 exists between these sequences, support identification of this insertion as an alternatively spliced exon. To confirm the existence of these two (FcγRIIb1 and IIb2) alterna-

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fcrIIb3  gccctctagggtagaatcgccaagctttgagagaaggctgt*************************
fcrIIb2                                            gactgctgtgctctgggcgccagc  65

fcrIIb3  *******************************************************************
fcrIIb2  tcgctccagggagtgATGGGAATCCTGTCATTCTTACCTGTCCTTGCCACTGAGAGTGACTGGGC  130

fcrIIb3  *************************************************************----
fcrIIb2  TGACTGCAA********************************************************
fcrIIb1  *********GTCCCCCCAGCCTTGGGGTCATATGCTTCTGTGGACAGCTGTGCTATTCCTGGCTC  195

fcrIIb3  ------------------*******************************************
fcrIIb2  *******************************************************
fcrIIb1  CTGTTGCTGGGACACCTGCAGCTCCCCCAAAGGCTGTGCTGAAACTCGAGCCCCAGTGGATCAAC  260

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  GTGCTCCAGGAGGACTCTGTGACTCTGACATGCCGGGGGACTCACAGCCCTGAGAGCGACTCCAT  325

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  TCAGTGGTTCCACAATGGGAATCTCATTCCCACCCACACGCAGCCCAGCTACAGGTTCAAGGCCA  390

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  ACAACAATGACAGCGGGGAGTACACGTGCCAGACTGGCCAGACCAGCCTCAGCGACCCTGTGCAT  455

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  CTGACTGTGCTTTCTGAGTGGCTGGTGCTCCAGACCCCTCACCTGGAGTTCCAGGAGGGAGAAAC  520

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  CATCGTGCTGAGGTGCCACAGCTGGAAGGACAAGCCTCTGGTCAAGGTCACATTCTTCCAGAATG  585

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  GAAAATCCAAGAAATTTTCCCGTTCGGATCCCAACTTCTCCATCCCACAAGCAAACCACAGTCAC  650

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  AGTGGTGATTACCACTGCACAGGAAACATAGGCTACACGCTGTTCTCATCCAAGCCTGTGACCAT  715

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  CACTGTCCAAGCTCCCAGCTCTTCACCGATGGGGATCATTGTGGCTGTGGTCACTGGGATTGCTG  780

fcrIIb3  *******************************************************
fcrIIb2  **************************************----
fcrIIb1  TAGCGGCCATTGTTGCTGCTGTAGTGGCCTTGATCTACTGCAGGAAAAAGCGGATTTCAGCTCTC  845

fcrIIb3  *******************************************************
fcrIIb2  ---------------------------------*************
fcrIIb1  CCAGGATACCCTGAGTGCAGGGAAATGGGAGAGACCCTCCCTGAGAAACCAGCCAATCCCACTAA  910

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  TCCTGATGAGGCTGACAAAGTTGGGGCTGAGAACACAATCACCTATTCACTTCTCATGCACCCGG  945

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  ATGCTCTGGAAGAGCCTGATGACCAGAACCGTATTTAGtctctccattgtcttgcattgggatttga  1040

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  gaagaaaatcagagagggaagatctggtatttcctggcctaaattcccccttggaggacagggaga  1105

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  tgctcgagttccaaaagagaaggtttcttccagagtcatctacctgagtcctgaagctccctgtc  1170

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  ctgaaagccacagacaatatggtcccaaataaccgactgcacctgctgtcttcagctcttcttga  1235

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  catcaaggctcttccgttccacatccacacagccaatccaattaatcaaaccactgttattaaca  1300

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  gataatagcaacttgggaaatgcttatgttacaggttaccgttgagaacaatcatctaaatctat  1365

fcrIIb3  *******************************************************
fcrIIb2  *******************************************************
fcrIIb1  atgatttcagaaatgttaaaatagactaacctctaccagcacattaaaagtgattgtttctgggt  1430

fcrIIb3  *******************************************
fcrIIb2  *******ttattgatgatttttattttctttattttctataaagatcatatattacttttaata  1495
fcrIIb1  gataaaaaaaaaaaaaaaaaaaaaa

fcrIIb2  aaaa                                                              1500
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FIGURE 3. Nucleotide sequences of three alternatively spliced forms of FcγRIIb cDNAs. Numbers in the right hand margin are arbitrary in that they denote positions in the composite sequence only and do not correspond to a particular cDNA. Untranslated regions are indicated in lower case while coding regions are shown in upper case. Asterisks indicate identity with the nucleotide below, while hyphens indicate the absence of a nucleotide in an alternatively spliced exon. The consensus polyadenylation site is underlined. These sequence data have been submitted to the EMBL/GenBank Data Libraries.

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fcrIIb3  **********************************************--------*********************
fcrIIb2  MGILSFLPVLATESDWADC*******************************************************
fcrIIb1                      KSPQPWGHMLLWTAVLFLAPVAGTPAAPPKAVLKLEPQWINVLQED  21
                                             +1

fcrIIb3  ********************************************************************************
fcrIIb2  ********************************************************************************
fcrIIb1  SVTLTQRGTHSPESDSIQWFHNGNLIPTHTQPSYRFKANNDSGEYIQTGOTSLSDPVHLTVLS  86

fcrIIb3  ********************************************************************************
fcrIIb2  ********************************************************************************
fcrIIb1  EWLVLQTPHLEFQEGETIVLRQHSWKDKPLVKVTFFQNGKSKKFSRSDFNFSIPQANHSHSGDYH  151

fcrIIb3  ********************************************************************************
fcrIIb2  ********************************************************-----------
fcrIIb1  QRGNIGYTLFSSKPVTITVQAPSSSPMGIIVAVVTGIAVAAIVAAVVALIYCRKKRISALPGYPE  216

fcrIIb3  **************************************************
fcrIIb2  -----------------*************************************
fcrIIb1  CREMGETLPEKPANPTNPDEADKVGAENTITYSLLMHPDALEEPDDQNRI  266
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FIGURE 4. Amino acid sequences predicted by the three alternatively spliced forms of FcγRIIb cDNAs are presented in the one letter code. Numbers in the right hand margin denote amino acid positions of FcγRIIb1. Asterisks indicate identity with the amino acid below and hyphens indicate a missing residue from an alternatively spliced exon. A 44 amino acid signal sequence with an underlined hydrophobic core is predicted preceding position +1. Cysteine residues that are predicted to form disulfide bonds are circled. Sites for N-linked glycosylation are boxed. The 23 amino acid hydrophobic core of the transmembrane domain is underlined.

tively spliced messages in authentic cellular RNA, FcγRIIb-specific oligonucleotide primers were annealed to RNA extracted from myeloid and lymphoid cells, converted to cDNA by reverse transcription and the resulting DNA stand was amplified by the PCR. Sequence analysis of these cDNAs confirmed the existence of alternatively spliced messages that correspond precisely to FcγRIIb1 and IIb2 (not shown). FcγRIIb1 ends with 19 A residues that are not encoded in the known FcγRIIb gene. This sequence presumably represents a poly(A) tail in the mRNA that gave rise to this clone, although no consensus polyadenylation signal is found in preceding sequences (discussed below).

The cDNA termed FcγRIIb3 in Figs. 1 and 3 contains an open reading frame of 931 nucleotides beginning at position 81 that predicts a mature protein of 29,277 daltons (like FcγRIIb1). The feature that distinguishes FcγRIIb3 from previously discussed cDNAs of this subclass is the deletion of 21 nucleotides (193–213 in Fig. 3) that predict the last seven amino acids of the signal sequence (Fig. 4). Genomic sequence analysis indicates that the missing nucleotides correspond precisely to an exon that is located between exons encoding the NH2-terminal sequences of the signal sequence and the first extracellular domain, suggesting that this difference results from an alternative splicing event. It should be noted that the exon that is spliced out in FcγRIIb3 encodes amino acids contributing to the hydrophobic core of the signal sequence of a nascent FcγRIIb protein (Fig. 4). A similar situation has been described for transcripts of the α subunit gene of the rat high affinity IgE receptor. A 21-bp exon encoding the COOH-terminal sequences of the signal sequence undergoes alternative splicing, as defined by cDNA analysis of mast cell RNA (23).

These studies demonstrate that primary transcripts from the FcγRIIb gene undergo alternative splicing at both the 5' end to generate FcγRIIb3 and at the 3' end to generate the alternative cytoplasmic domains exemplified by FcγRIIb1 and IIb2.

In addition to these clones containing complete coding sequences, we have isolated a truncated FcγRIIb cDNA that lacks sequences encoding the cytoplasmic region of these receptors. This truncated clone is identical to FcγRIIb2 from the 5' untranslated region through sequences predicting the transmembrane domain (po-

sitions 67–840 in Fig. 3) at which point it diverges completely. The divergent sequence is identical to that of the genomic intron of this region and to that of the previously isolated cDNA of this subclass (16), suggesting that both cDNAs represent incompletely processed pre mRNAs.

Isolation and Analysis of FcγRIIa'.   A minimum of three distinct genes encode FcγRII transcripts. Overlapping cosmid clones encoding human FcγRII genes have been isolated and analyzed. The sequence of those clones confirms that FcγRIIa derives from one gene and that the FcγRIIb sequences (1, 2, and 3) are derived from alternative splicing of a second gene (Qiu, W. Q., and J. V. Ravetch, unpublished observations). A third FcγRII gene was identified that is composed of the 5' exons of an FcγRIIb-like gene (5' untranslated, signal, extracellular, and transmembrane) and the 3' exons of an FcγRIIa-like gene (cytoplasmic and 3' untranslated; Qiu, W. Q., and J. V. Ravetch, unpublished observations). This gene is predicted to give rise to transcripts that are structurally distinct from the FcγRIIa and IIb molecules discussed above. A cDNA clone was isolated from a Daudi library and characterized. Its sequence indicated that it represented an incompletely processed transcript of this IIa' gene, lacking only the last cytoplasmic and 3' untranslated sequences. To test for the presence of a complete transcript, cellular RNAs were characterized by reverse transcription and PCR amplification using a 3' cytoplasmic domain oligonucleotide from FcγRIIa and a 5' untranslated region oligonucleotide from FcγRIIb. A cDNA product of the expected size and structure was obtained (Fig. 1) in neutrophil monocytic and B lymphoid cell RNAs as confirmed by hybridization to domain specific probes (data not shown). The sequence of IIa' is >99% identical to IIb from the 5' untranslated through the transmembrane domain. The cytoplasmic and 3' untranslated sequences, however, are >95% identical to IIa (data not shown).

Sequence Homology between Murine and Human FcγRIIs.   Comparisons of human and mouse FcγRII cDNAs shown in Fig. 5 (nucleotide) and Table I (amino acid) reveal both striking homology and significant differences. Dot matrix comparison of the human FcγRIIb2 cDNA sequence with that of its murine homologue (FcγRβ2) shows that they are related throughout their length, as shown in Fig. 5 D. From a high of 75% in the extracellular region, these sequences retain >50% nucleotide identity in 5' and 3' noncoding regions. In a similar fashion, comparison of human FcγRIIb1 with its murine homologue FcγR β1 shows the close relationship between these gene products. However, a deletion of 81 nucleotides in the cytoplasmic domain of FcγRIIb1 is apparent relative to its murine counterpart (Fig. 5 C). In contrast, comparison of human FcγRIIa and murine FcγR α (Fig. 5 B, Table I) reveals a markedly different pattern. Those transcripts demonstrate homology in nucleotide sequences predicting the signal sequence and extracellular domains. However, the transmembrane, cytoplasmic, and 3' untranslated regions of these two clones share no homology.

The evolutionary relationship of FcγRIIa/a' with its murine counterpart is evident from the two regions of homology that are found between human FcγRIIa/a' and IIb (or murine β, not shown) (Fig. 5 A). The first region begins with nucleotide sequences that predict the extracellular region and continues through the membrane spanning domain and into the first portion of the cytoplasmic region (Table I). A 1-kb insertion follows in the FcγRIIa/a' sequence for which no homology in FcγRIIb is apparent. The second homology region begins in the 3' noncoding region of
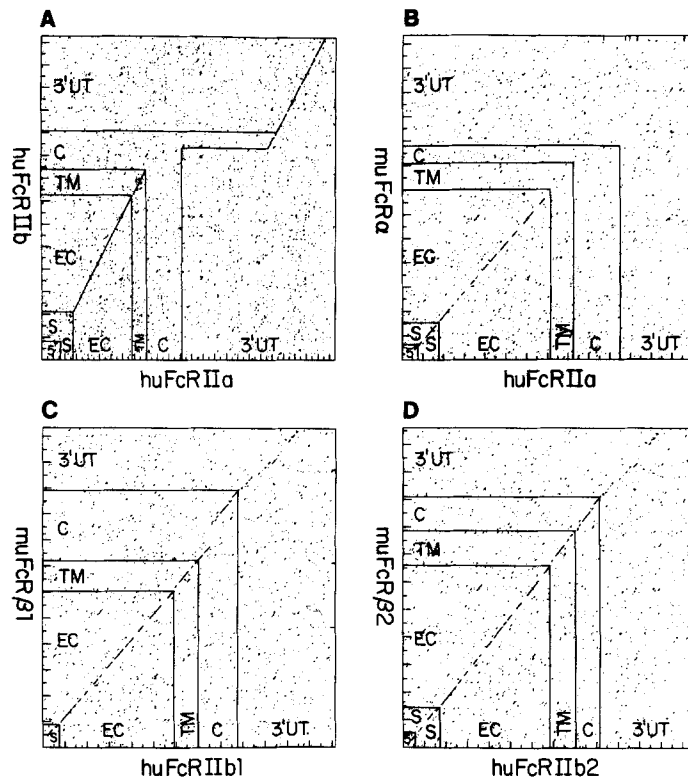
FIGURE 5. Dot matrix comparison of FcγR cDNA sequences of human and mouse using the DIAGON program (24). Complete nucleotide sequences of human (hu; this article) and murine (mu, reference 4) FcγRs are compared along the x- or y-axis as indicated. The 5' untranslated (5'), signal sequence (S), extracellular (EC), transmembrane (TM), cytoplasmic (C), and 3' untranslated regions are indicated in letters and demarcated by horizontal and vertical solid lines. Regions of nucleotide identity appear as dots and diagonal lines. Insertions of nucleotide sequence appear as gaps in the diagonal line. Full size scale bars denote spacing of 100 base pairs on all axes. (A) Comparison of human FcγRIIa and IIb1 cDNAs. (B) Comparison of human FcγRIIa and murine FcγR α. (C) Comparison of human FcγRIIb1 and murine FcγR β_1. (D) Comparison of human FcγRIIb2 and murine FcγR β_2.

TABLE I

Amino Acid Sequence Homology Between Human and Mouse FcγRII

FcγR	S	EC	TM	C
		%		
IIa:IIb2	17	96	88	<15*
IIa:α	55	59	<15	<15
IIa:β2	23	61	50	<15*
IIb:α	<15	60	<15	<15
IIb1:β1	48	63	64	60
IIb2:β2	48	63	64	53
α:β	<15	95	<15	<15

* huFcγRIIa cytoplasmic domain is encoded by 2 exons, the shorter NH2-terminal exon is 60% identical to huFcγRIIb2 and 37% identical to moFcγRβ2. The longer COOH-terminal region is unrelated to either of these cDNAs resulting in a nonsignificant overall homology.

FcγRIIa/a′ and in sequences encoding the cytoplasmic domain of FcγRIIb2 and extends through the end of both clones (Fig. 5 A, see Discussion). In view of the extensive homology between FcγRIIb2 and its murine homologue β_2 (Fig. 5 D) the latter cDNA also shares a strong homology with the 3′ untranslated region of FcγRIIa/a′. FcγRIIa, though not IIa′, has a chimeric organization, with distinct regions that are homologous to either of the two murine FcγRs. The FcγRIIa signal sequence is homologous only to the murine α FcγR (Fig. 5 B), with both IIa and IIa′ demonstrating homology in their extracellular region to both murine α and β sequences, while their transmembrane, some of the cytoplasmic and 3′ untranslated regions are homologous to the murine β_2 FcγR or its human homologue FcγRIIb2 (Table I and Fig. 5 A).

FcγRs Display Distinct Patterns of mRNA Expression. To determine if these structurally distinct molecules displayed different patterns of mRNA expression, as in the mouse (4), oligonucleotide probes to the signal sequences of FcγRIIa and IIb were used to reveal distinct patterns of mRNA expression by Northern analysis. FcγRIIa transcripts are expressed in monocyte-like cell lines (Fig. 6 A, lanes 3-7), HL-60 differentiated with DMSO (Fig. 6 A, lane 2), and in the erythroleukemic cell line K562 (Fig. 6 A, lane 1). Four lymphoid cell lines do not express FcγRIIa mRNA (Fig. 6 A lanes 9-12) although the Burkitt lymphoma cell line Daudi does (Fig. 6
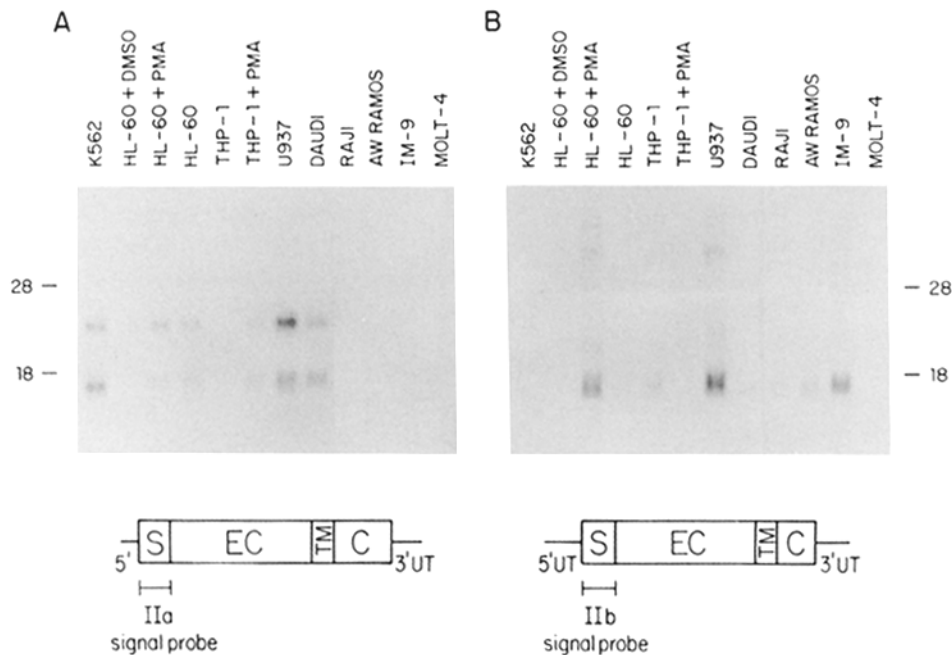
FIGURE 6. Northern blot analysis of human FcγRII RNA expression. 2 μg of poly(A)⁺ RNA was prepared from human cell lines described in Materials and Methods and electrophoresed in a formaldehyde agarose gel and transferred to nitrocellulose. (A) The filter was hybridized with a FcγRIIa signal probe indicated schematically below the autoradiogram. (B) After removal of the probe and preexposure the filter was rehybridized to a FcγRIIb signal probe as indicated. RNA concentration was normalized by ethidium bromide staining and hybridization with actin.

A, lane *8*). As shown in Fig. 6 *A*, the human FcγRIIa–specific signal probe reveals two bands of 2.6 and 1.5 kb in all positive cell lines. A cDNA probe to sequences 3' of the first polyadenylation signal of FcγRIIa (see Figs. 1 and 2) hybridized only to the 2.6-kb transcript (data not shown), suggesting that these two transcripts arise from differential polyadenylation. In contrast to IIa, the FcγRIIb signal probe detects a band of 1.5–1.6 kb that is expressed in numerous lymphoid and myeloid cell lines as shown in Fig. 6 *B* (lanes *3–11*). Among cell lines that have been tested, only K562 (lane *1*), MOLT-4 (lane *12*), and DMSO-treated HL-60 (lane *2*) do not express FcγRIIb mRNA. However, the IIb signal probe cannot distinguish between IIa' and IIb since the predicted FcγRIIa' transcript, identified in neutrophils, monocytes, and lymphocytes, possesses a FcγRIIb-like signal. These transcripts can, however, be distinguished using PCR and distinct combinations of oligonucleotides as described above (Materials and Methods). In this manner, IIa' expression has been detected in neutrophils, B lymphocytes, cultured adherent monocytes, U937 cells, and placenta, but not in T lymphoid cell line RNAs (data not shown). To confirm results obtained with cell lines total RNAs from primary human cells were analyzed for FcγRII expression (not shown). Neutrophils, cultured adherent monocytes, and chronic myelogenous leukemia cells all expressed abundant FcγRIIa mRNA by Northern analysis. Human placenta, a rich source of monocyte and macrophage-like cells, expressed readily detectable FcγRIIa and IIb mRNA on Northern blots. In contrast FcγRIIb mRNA could only be detected in B lymphocytes, cultured adhert monocytes, and neutrophils by reverse transcription and PCR amplification of these primary cell RNAs.

Immune Complexes of IgG and mAbs Bind Human FcγRII Expressed by Gene Transfer.   The ligand binding properties and epitopes of FcγRII proteins predicted by these cDNAs were determined by the expression of these clones in FcγR⁻ fibroblast cells. Transient transfection of murine Ltk⁻ fibroblasts with FcγRII cDNAs cloned into an SV40-based vector (pCEXV-3) resulted in cell surface expression of all FcγRs discussed above. Positive transfectants were detected by binding of mouse IgG₁ opsonized SRBCs indicating the capacity of these FcγRs to bind multivalent immune complexes (Fig. 7). FcγRIIa (Fig. 7 *A*) demonstrates a consistently decreased level of mouse IgG1 rosetting. This difference is not due to a difference in the efficiency of cell surface expression of the protein encoded by this cDNA, since rosetting with mAb IV.3 (20)-coupled erythrocytes gave equivalent densities of bound cells (not shown). Rather, these data suggest that FcγRIIa has a decreased affinity for mouse IgG1 compared with FcγRIIb. FcγRIIb3, lacking part of the hydrophobic core of its signal sequence, yields transfectants with an equivalent density of bound mouse IgG1- or CD32 mAb–coated SRBCs, indicating the functional integrity of the surface FcγRII molecule. The functional consequences of this alternative splice is not apparent and is under investigation.

Characterization of these sequences as encoding authentic FcγRII proteins was determined by epitope mapping using well-characterized mAbs (20–22) that define this class of molecules. Murine fibroblasts stably expressing human FcγRs were assayed by indirect immunofluorescence and flow cytometry for recognition by anti-FcRII mAbs. All CD32 mAbs (22) and the mAbs IV.3 (20) and KuFc79 (21) recognized both FcγRIIa and IIb transfectants (not shown).
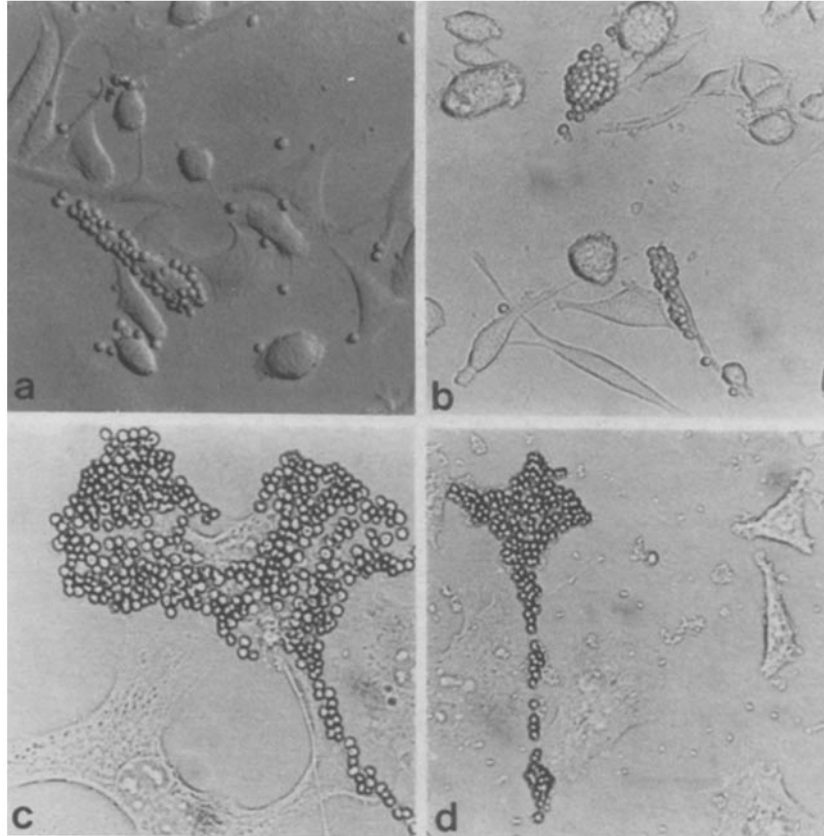
FIGURE 7. Rosetting assay of human FcγRII expressing transfected cells. FcR⁻ mouse LTK⁻ fibroblasts were transiently transfected with the indicated human FcγRII cDNA in the expression vector pCEXV-3. 2 d after transfection, positive cells were detected by binding of mouse IgG1 opsonized SRBCs. Plates were washed of unbound SRBCs, fixed in glutaraldehyde, and photographed. (a) IIa, (b) IIb1, (c) IIb2, (d) IIb3.

Discussion

In this article we establish the structural heterogeneity of human FcγRII molecules. Previous studies using IgG affinity binding and an anti-FcγRII mAb (20) suggested that this class of molecules was more heterogeneous than could be accounted for by the expression pattern of the previously isolated FcγRII cDNA (14–16; this article). Cloning of multiple cDNAs representing alternatively spliced transcripts of the FcγRIIb gene and identification of the IIa′ transcript complement the previously isolated cDNA as subclasses of this important class of receptors. Overlapping yet distinct subsets of cell lines express mRNAs encoding these receptors. This pattern of RNA expression is consistent with the distribution of FcγRII protein determined by mAb and ligand binding studies. Both a and b subclasses of FcγRII have been shown by gene transfer to bind immune complexes and be recognized by anti-

FcRII mAbs. Together these three subclasses account for all ligand and mAb binding attributed to human FcRII to date.

Human FcγRIIa, IIa' and IIb cDNAs predict proteins that are closely related yet have important structural differences. In particular, the mature proteins predicted by FcγRIIa and IIa' genes are >95% identical throughout. Receptors predicted by the FcγRIIb cDNAs share this homology with IIa/IIa' in the extracellular domain accounting for their ability to bind common ligands and mAbs. Unlike their murine counterparts the human receptors display high homology in their transmembrane domains (Table I). While 10 of the first 12 residues of their intracellular regions are identical, FcγRIIa/a' and IIb2 diverge completely at that point. The largely divergent cytoplasmic tails of these receptors may mediate distinct functions in response to binding a common ligand.

Both conservation and divergence in primary structure of members of the murine and human FcγR multigene families lead to important predictions about their functions. A striking conservation of sequence is observed between human FcγRIIb and murine FcγR β. A human gene encoding FcγRIIb has an identical exon-intron organization to that of the FcγR β gene (Qiu, W. Q., and J. V. Ravetch, unpublished observations). In both species there is alternative splicing of the primary transcripts from these genes that involves their first cytoplasmic exons. The human FcγRIIb2 and murine FcγR β2 cDNAs lacking these exons are homologous throughout their coding and noncoding regions (Fig. 6 D). In contrast, the cDNA molecules including these exons reveal an interspecies divergence (Fig. 6 C). In human FcγRIIb1 there is a deletion of sequences corresponding to the COOH-terminal 27 amino acids of the first cytoplasmic exon of murine FcγR β1, a result of the utilization of a cryptic splice donor site in this sequence. Surprisingly, this same sequence AGGTGAGT is present in the murine homologue (4), yet its use as a splice donor has not been detected.

In contrast, a direct relationship between human FcγRIIa, IIa', and a single murine counterpart is not obvious. Analysis of both cDNA and genomic sequence data for FcγRIIa and IIa' indicates that the sequence preceding the second region of homology between FcγRIIa/a' and murine FcγR β2 (or human FcγRIIb1, Fig. 5 A) could be a mutated splice acceptor site. In particular, a T residue inserted into the critical AG splice acceptor dinucleotide (Fig. 8) would have rendered that site nonfunctional. With the utilization of a cryptic splice site in the intron preceding this site, these sequences persist as exon in FcγRIIa/a' transcripts. Therefore, the nucleo-
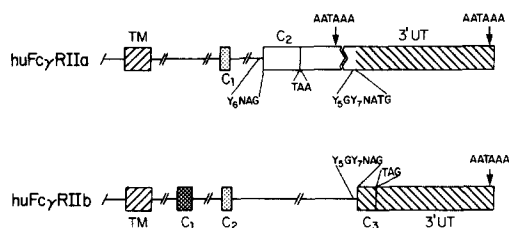

Figure 8.   Schematic comparison of the 3' ends of human FcγRII genes. Sequences contributing to cDNAs reported here are boxed and labeled as in Fig. 1. Intron sequences are indicated by broken horizontal lines. Cross-hatching and stippling indicate regions of high homology. Relevant nucleotide sequences are indicated including: polyadenylation signals, stop codons, and splice acceptor sites, Y, pyrimidine; N, any nucleotide. The T residue that is proposed to have mutated a splice acceptor site in the FcγRIIa gene is shown in bold. The structure of the IIa' gene is identical to IIa in this region.

tide sequences for both coding regions and 3' untranslated sequences of murine FcγR β and human FcγRIIb (Fig. 5 A) are homologous to the 3' untranslated region of human FcγRIIa/a' because of the presence of the ancestral cytoplasmic/3' untranslated exon in the latter sequence (Fig. 8). The novel exon found in the FcγRIIa/a' gene likely results from these events having converted sequences 3' of the new acceptor site, which are intron sequences in IIb and mouse β, to exon sequences in IIa/a'. Thus, IIa' most likely arose from an ancestral IIb gene by mutation of a splice acceptor site and utilization of a cryptic site. The persistence of those sequences as an open reading frame for the cytoplasmic domain of the human IIa/a' transcript suggests the acquisition of a functional role for these sequences. The chimeric structure of FcγRIIa noted above (Fig. 5, A and B) suggested that the transcript from which this cDNA was obtained is the product of a gene that arose by recombination between two genes that were precursors of the present day murine FcγRα and β genes. Such an event would explain the murine FcγR α-like signal sequence and murine FcγRβ2-(or human FcγRIIb2) like transmembrane, first cytoplasmic portion, and 3' untranslated regions noted above and by others (16).

Selective functions for FcγRs IIa, IIa', and IIb are further suggested by the cell type–specific expression of these three genes. RNA expression from these genes has been distinguished through use of signal sequence probes that are specific for FcγRIIa and FcγRs IIa'/IIb and by the use of reverse transcription and PCR to distinguish IIa' from IIb. Monocyte/macrophage–like human cells including cultured adherent monocytes and cell lines such as U937 express all three subclasses of FcγRII. Peripheral blood neutrophils express readily detectable FcγRIIa mRNA with a lower level of FcγRIIb and FcγRIIa' mRNA. Splenic B lymphocytes and cell lines of B lymphoid origin (IM-9, Daudi, Raji, AW Ramos) all express FcγRIIb mRNA, a finding that is consistent with the ubiquitous lymphocyte expression of the murine homologue, FcγR β (4). Three human T cell lines (MOLT-4, Jurkat, and Fro-2) fail to express detectable levels of human FcγRII mRNA. While these patterns of RNA expression are consistent with previous studies of RNA (14–16) and protein expression (reviewed in references 1 and 9) for FcγRII (CD32), those studies failed to distinguish between subclasses of the FcγRII(CD32) family as demonstrated here. Recognition of the structural differences among these molecules and their specificities of cellular expression suggest functionally important distinctions.

Summary

The structural heterogeneity of the human low affinity receptor for IgG, FcRII(CD32), has been elucidated through the isolation, characterization, and expression of cDNA clones derived from myeloid and lymphoid RNA. These clones predict amino acid sequences consistent with integral membrane glycoproteins with single membrane spanning domains. The extracellular domains display sequence homology to other FcγRs and members of the Ig supergene family. A minimum of three genes (FcγRIIa, IIa', and FcγRIIb) encode these transcripts, which demonstrate highly related extracellular and membrane spanning domains. IIa/IIa' differ substantially in the intracytoplasmic domain from IIb. Alternative splicing of the IIb gene generates further heterogeneity in both NH_2- and COOH-terminal domains of the predicted proteins. Comparison to the murine homologues of these molecules reveals a high degree of conservation between the products of one of these

genes, FcγRIIb, and the murine β gene in primary sequence, splicing pattern, and tissue distribution. In contrast, the sequence of IIa' indicates its relationship to the β-like genes, with mutation giving rise to a novel cytoplasmic domain, while IIa is a chimera of both α- and β-like genes. Expression of these cDNA molecules by transfection results in the appearance of IgG binding molecules that bear the epitopes defined by the FcRII(CD32) mAbs previously described.

Note added in proof: cDNA clones essentially identical to IIa' and IIb2 have recently been described (25).

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