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Immunology

Molecular cloning of a human immunoglobulin G Fc receptor

(antigen–antibody receptor/sequence homology/domain structure/immunoglobulin gene superfamily)

MARGARET L. HIBBS, LISA BONADONNA, BERNADETTE M. SCOTT, IAN F. C. MCKENZIE, AND P. MARK HOGARTH*

Research Centre for Cancer and Transplantation, Department of Pathology, The University of Melbourne, Parkville, Victoria, 3052, Australia

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ABSTRACT **Human IgG Fc receptor (FcγR) cDNA clones were isolated by cross-species hybridization by probing cDNA libraries with the low-affinity FcγR β1 cDNA clone from mouse as well as a pool of oligonucleotides constructed from the nucleotide sequence of this FcγR. Three cDNA clones were isolated and analysis of the predicted amino acid sequence indicated that the human FcγR protein is synthesized with a 34-amino acid leader and the mature protein is composed of 281 amino acids. The extracellular region of this FcγR was divided into two domains, which were very similar to each other and to the corresponding regions of both mouse α and β FcγRs and showed a clear relationship to immunoglobulin variable regions. One possible N-linked glycosylation site was found in each of the extracellular domains. The human FcγR leader sequence was shown to be similar to the mouse α FcγR leader sequence, but the transmembrane region was most similar to the mouse β1 FcγR. The intracellular domain of the human FcγR was surprisingly different from both mouse FcγRs. RNA blot analysis of human cells demonstrated two transcripts (2.5 and 1.5 kilobases) that arise by use of different adenylylation signals. The cellular expression of these transcripts suggests that they encode the low-affinity p40 FcγR protein.**

Receptors for the Fc portion of IgG (FcγRs) play an essential role in the protection of an organism against foreign antigens. These receptors are present on monocytes, macrophages, neutrophils, natural killer (NK) cells, and T and B lymphocytes, and they participate in diverse functions such as phagocytosis of immune complexes and modulation of antibody production by B cells (1, 2). Three classes of human FcγR receptors have been defined and can be distinguished by several criteria, including differential reactivity with monoclonal antibodies (mAbs), distinct biochemical properties, and differences in affinity of the receptors for immunoglobulin subclasses (2). The high-affinity receptor, detected by mAb 32, has an average molecular mass of 72 kDa (2, 3), whereas the two distinct low-affinity receptors, FcγRII defined by mAb IV*3* and $FcγR_{lo}$ (CD16) defined by mAb 3G8, have different molecular masses of 40 kDa and 50–70 kDa, respectively (4, 5). Human leukocyte FcγRs also differ in specificity of IgG subclass binding (2). Receptors with different subclass specificities have been described in the mouse (1) and the recent isolation of three unique mouse FcγR cDNAs indicates that this observed subclass specificity may be due to the presence of structurally related but distinct mouse FcγR proteins (6–9).

To define the structural and functional characteristics of the different human FcγRs, cDNA clones have been isolated and characterized.†

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METHODS

Screening of Human cDNA Libraries. Two human cDNA libraries were used: (*i*) human monocyte cDNA λgt10 library (Clontech Laboratories, Palo Alto, CA) and (*ii*) phytohemagglutinin-stimulated human peripheral blood leukocyte cDNA λgt10 library (obtained from T. Mak, Cancer Institute of Ontario, Toronto). The monocyte cDNA library was plated on *Escherichia coli* C600/*Hfl* and 2×10^5 plaque-forming units were screened in duplicate with the mouse β1 FcγR cDNA and a pool of three oligonucleotides constructed from the nucleotide sequence of the mouse β1 FcγR cDNA. This pool consisted of three probes, which corresponded to the sequences encoding the N terminus (nucleotides 137–185), the second extracellular domain (nucleotides 545–574), and the C terminus (nucleotides 956–1000). Plaques were lifted onto nylon filters (Hybond N, Amersham International, U.K.), fixed by ultraviolet light irradiation, and hybridized overnight in 20% (vol/vol) formamide/5× SSC (0.75 M NaCl/75 mM sodium citrate)/0.1% $NaDodSO_4$/20 mM phosphate buffer, pH 6.8/0.125% non-fat dry milk at 35°C. Washing was carried out at low stringency in 1× SSC/0.1% $NaDodSO_4$ at 35°C. Oligonucleotides were end-labeled with [^{32}P]ATP (10) and cDNA probes were labeled to a high specific activity by random priming using hexamers (Pharmacia, Uppsala, Sweden; ref. 11). The phytohemagglutinin-stimulated peripheral blood leukocyte library was screened with the insert from a cDNA clone isolated from the human monocyte cDNA library using hybridization conditions as described above. DNA was prepared from clonal bacteriophage (12) and the cDNA inserts were purified and subcloned into pJL4 (13) for subsequent DNA production.

Nucleotide Sequencing. DNA sequence was determined by the dideoxynucleotide chain-termination method (14) after directional or random subcloning of fragments into the sequencing vectors M13mp18 or M13mp19 as outlined in Fig. 1*a*. Nucleotide and amino acid sequences were analyzed by the MELBDBSYS (Melbourne University) and the Dayhoff ALIGN program (15).

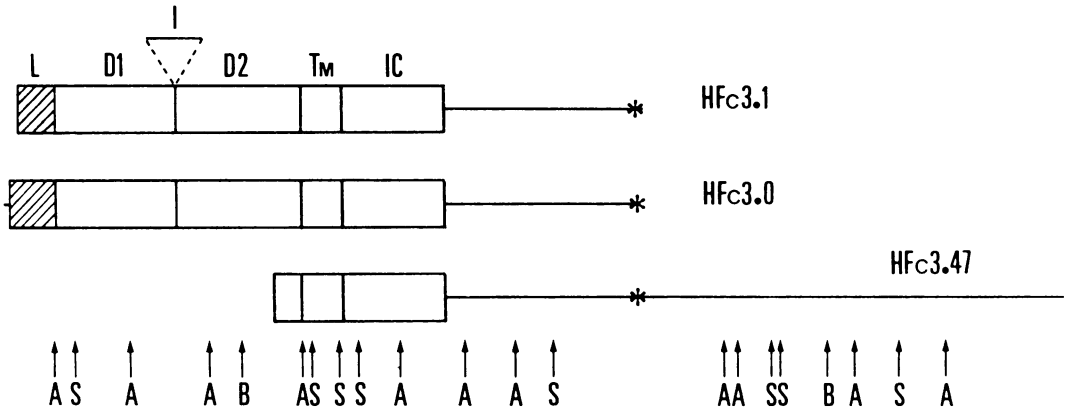
RNA Blot Analysis. Poly(A)$^+$ RNA was prepared from normal human spleen by the guanidinium isothiocyanate method and chromatography on oligo(dT)-cellulose (16, 17). Poly(A)$^+$ RNA (5 μg) was electrophoresed in 1% agarose-formaldehyde gels and transferred to Hybond N (Amersham) as described (10). Hybridization with the human FcγR cDNA probe was carried out overnight in 50% (vol/vol)

Abbreviations: FcγR, receptor for the Fc portion of IgG; mAb, monoclonal antibody; ORF, open reading frame; UTR, untranslated region; FcγRII, low-affinity p40 human FcγR protein; $FcγR_{lo}$, low-affinity p50-70 human FcγR protein; FcγRI, high-affinity p72 human FcγR protein.

*To whom reprint requests should be addressed.

†This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03619).

a



b

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          -34                 -30                                 -20                                 -10
          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 Ala
GAATTCCAACT 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 GCT    92
                      -1 ▼+1                              10                                  20
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
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   182
          ●           30                                  40                                  50
Leu Thr Cys Gln Gly Ala Arg Ser Pro Glu Ser Asp Ser Ile Gln Trp Phe His Asn Gly Asn Leu Ile Pro Thr His Thr Gln Pro Ser
CTG ACA TGC CAG GGG GCT CGC AGC CCT GAG AGC GAC TCC ATT CAG TGG TTC CAC AAT GGG AAT CTC ATT CCC ACC CAC ACG CAG CCC AGC   272
                      60  *                   ●              70                                  80
Tyr Arg Phe Lys Ala Asn Asn Asn Asn Asp Ser Gly Glu Tyr Thr Cys Gln Thr Gly Gln Thr Ser Leu Ser Asp Pro Val His Leu Thr Val
TAC AGG TTC AAG GCC AAC AAC AAT GAC AGC GGG GAG TAC ACG TGC CAG ACT GGC CAG ACC AGC CTC AGC GAC CCT GTG CAT CTG ACT GTG   362
                      90                                  100                                 ●110
Leu Ser Glu Trp Leu Val Leu Gln Thr Pro His Leu Glu Phe Gln Glu Gly Glu Thr Ile Met Leu Arg Cys His Ser Trp Lys Asp Lys
CTT TCC GAA TGG CTG GTG CTC CAG ACC CCT CAC CTG GAG TTC CAG GAG GGA GAA ACC ATC ATG CTG AGG TGC CAC AGC TGG AAG GAC AAG   452
                      120                                 130                                 140      *
Pro Leu Val Lys Val Thr Phe Phe Gln Asn Gly Lys Ser Gln Lys Phe Ser Arg Leu Asp Pro Thr Phe Ser Ile Pro Gln Ala Asn His
CCT CTG GTC AAG GTC ACA TTC TTC CAG AAT GGA AAA TCC CAG AAA TTC TCC CGT TTG GAT CCC ACC TTC TCC ATC CCA CAA GCA AAC CAC   542
                      150 ●                               160                                 170
Ser His Ser Gly Asp Tyr His Cys Thr Gly Asn Ile Gly Tyr Thr Leu Phe Ser Ser Lys Pro Val Thr Ile Thr Val Gln Val Pro Ser
AGT CAC AGT GGT GAT TAC CAC TGC ACA GGA AAC ATA GGC TAC ACG CTG TTC TCA TCC AAG CCT GTG ACC ATC ACT GTC CAA GTG CCC AGC   632
                      180                                 190                                 200
Met Gly Ser Ser Ser Pro Met Gly Ile Ile Val Ala Val Val Ile Ala Thr Ala Val Ala Ala Ile Val Ala Ala Val Val Ala Leu Ile
ATG GGC AGC TCT TCA CCA ATG GGG ATC ATT GTG GCT GTG GTC ATT GCG ACT GCT GTA GCA GCC ATT GTT GCT GCT GTA GTG GCC TTG ATC   722
                      210                                 220                                 230
Tyr Cys Arg Lys Lys Arg Ile Ser Ala Asn Ser Thr Asp Pro Val Lys Ala Ala Gln Phe Glu Pro Pro Gly Arg Gln Met Ile Ala Ile
TAC TGC AGG AAA AAG CGG ATT TCA GCC AAT TCC ACT GAT CCT GTG AAG GCT GCC CAA TTT GAG CCA CCT GGA CGT CAA ATG ATT GCC ATC   812
                      240                                 250                                 260
Arg Lys Arg Gln Leu Glu Glu Thr Asn Asn Asp Tyr Glu Thr Ala Asp Gly Gly Tyr Met Thr Leu Asn Pro Arg Ala Pro Thr Asp Asp
AGA AAG AGA CAA CTT GAA GAA ACC AAC AAT GAC TAT GAA ACA GCT GAC GGC GGC TAC ATG ACT CTG AAC CCC AGG GCA CCT ACT GAC GAT   902
                      270                                 280
Asp Lys Asn Ile Tyr Leu Thr Leu Pro Pro Asn Asp His Val Asn Ser Asn Asn ***
GAT AAA AAC ATC TAC CTG ACT CTT CCT CCC AAC GAC CAT GTC AAC AGT AAT AAC TAA AGAGTAACGTTATGCCATGTGGTCATACTCTCAGCTTGCTGAG  1002
TGGATGACAAAAAGAGGGGAATTGTTAAAGGAAAATTTAAATGGAGACTGGAAAAATCCTGAGCAAACAAAACCACCTGGCCCTTAGAAATAGCTTTAACTTTGCTTAAACTACAAACA  1121
CAAGCAAAACTTCACGGGGTCATACTACATACAAGCATAAGCAAAACTTAACTTGGATCATTTCTGGTAAATGCTTATGTTAGAAATAAGACAACCCCAGCCAATCACAAGCAGCCTAC  1240
TAACATATAATTAGGTGACTAGGGACTTTCTAAGAAGATACCTACCCCCAAAAAACAATTATGTAATTGAAAACCAACCGATTGCCTTTATTTTGCTTCCACATTTTCCCAATAAATAC  1359
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TGGTGCTATCTTGGCTCACTGCAAACCCGCCTCCCAGGTTTAAGCGATTCTCATGCCTCAGCCTCCCAGTAGCTGGGATTAGAGGCATGTGCCATCATACCCAGCTAATTTTTGTATTT  1597
TTTATTTTTTTTTTTTAGTAGAGACAGGGTTTCGCAATGTTGGCCAGGCCGATCTCGAACTTCTGGCCTCTAGCGATCTGCCCGCCTCGGCCTCCCAAAGTGCTGGGATGACCAGCATC  1716
AGCCCCAATGTCCAGCCTCTTTAACATCTTCTTTCCTATGCCCTCTCTGTGGATCCCTACTGCTGGTTTCTGCCTTCTCCATGCTGAGAACAAAATCACCTATTCACTGCTTATGCAGT  1835
CGGAAGCTCCAGAAGAACAAAGAGCCCAATTACCAGAACCACATTAAGTCTCCATTGTTTTGCCTTGGGATTTGAGAAGAGAATTAGAGAGGTGAGGATCTGGTATTTCCTGGACTAAA  1954
TTCCCCTTGGGGAAGACGAAGGGATGCTGCAGTTCCAAAAGAGAAGGACTCTTCCAGAGTCATCTACCTGAGTCCCAAAGCTCCCTGTCCTGAAAGCCACAGACAATATGGTCCCAAAT  2073
GACTGACTGCACCTTCTGTGCCTCAGCCGTTCTTGACATCAAGAATCTTCTGTTCCACATCCACACAGCCAATACAATTAGTCAAACCACTGTTATTAACAGATGTAGCAACATGAAAG  2192
ACGCTATGTTACAGGTTACATGAGAGCAATCATGTAAGTCTATATGACTTCAGAAATGTTAAAATAGACTAACCTCTAACAACAAATTAAAAGTGCGGAATTC                  2295
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FIG. 1. Restriction map, nucleotide sequence, and deduced amino acid sequence of the human FcγR. (*a*) Schematic representation of the human FcγR cDNA clones HFc3.1, HFc3.0, and HFc3.47. Untranslated sequences are represented by a line, and coding sequences are boxed. Hatched region represents the putative leader sequence; unhatched area represents the sequence encoding the mature protein. The leader sequence (L), extracellular domain 1 (D1), extracellular domain 2 (D2), transmembrane segment (Tm), intracellular domain (IC), and intervening sequence in HFc3.1 (I) are shown. The adenylylation signal sequence is marked with an asterisk. Restriction sites used in sequence determination are indicated as follows: A, *Alu* I; B, *Bam*HI; S, *Sau*3AI. *Eco*RI linkers were used for library construction and provided convenient restriction sites at the ends of all cDNA clones. All restriction sites were sequenced across and both strands were fully sequenced. (*b*) Nucleotide and predicted amino acid sequence of the human FcγR compiled from the sequences of HFc3.1, HFc3.0, and HFc3.47. Nucleotides are numbered at the end of each line. Untranslated sequence is in closed up type. The polyadenylylation signal sequence is boxed. The translated sequence is found above the nucleotide sequence and amino acids are numbered above the line commencing at the amino-terminal residue. The leader sequence is numbered from residue −34 to −1 and the amino terminus is indicated by arrowheads. The single hydrophobic transmembrane region is underlined by a solid line. Cysteine residues involved in disulfide bonding are marked with solid circles and asparagine residues possibly involved in attachment of carbohydrate are marked with asterisks.

formamide/5× SSPE/0.1% $NaDodSO_4$/0.125% non-fat dry milk at 55°C. Filters were washed in 0.2× SSPE/0.1% $NaDodSO_4$ at 55°C (1× SSPE = 180 mM NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA).

RESULTS

Isolation and Characterization of Human FcγR cDNA Clones. A human cDNA library derived from leukemic cells

of myeloid origin (THP-1; ref. 18) was used to isolate human FcγR cDNAs. Screening was performed at low stringency using the mouse β1 cDNA clone (low-affinity FcγR) and a pool of oligonucleotides constructed from the nucleotide sequence of the mouse β1 FcγR (see *Methods*). Two clones (λHFc3.1 and λHFc3.47) that hybridized with both the mouse cDNA and oligonucleotide probe pool were isolated and characterized. Phage DNA was prepared from both clones and after *Eco*RI digestion, λHFc3.1 and λHFc3.47 were found to contain 1.5-kilobase (kb) and 1.7-kb inserts, respectively. Southern hybridization analysis of *Eco*RI-digested λ DNA confirmed that the mouse β1 cDNA probe hybridized with both cDNA inserts. The cDNA inserts were then purified and characterized by restriction enzyme mapping and DNA sequencing (Fig. 1). The complete sequencing of HFc3.1 showed that it did not encode the entire FcγR protein and contained a 117-nucleotide insertion introducing an in-phase termination codon between the sequences encoding the extracellular domains (Fig. 1; see below). HFc3.47 encoded only part of the second extracellular domain as well as the transmembrane and cytoplasmic domains but extended the 3′ untranslated region 929 nucleotides. A third clone (λHFc3.0) was then isolated from a phytohemagglutinin-stimulated peripheral blood leukocyte cDNA library.

Nucleotide and Deduced Amino Acid Sequence of Human FcγR cDNA. The nucleotide sequence of human FcγR compiled from HFc3.1, HFc3.0, and HFc3.47 is shown in Fig. 1*b*. Clone HFc3.0 was composed of 1348 nucleotides. An open reading frame (ORF) of 945 nucleotides extended from nucleotides AUG at position 12 to the termination codon TAA at position 957. The nucleotide sequence of the ORF showed considerable similarity to coding sequences of mouse α and β1 cDNA (β1 was used to isolate these clones) and is discussed below (see also Table 1). A 3′ untranslated region (3′ UTR) of 402 nucleotides extends beyond the adenylylation signal AATAAA at position 1351; the poly(A) tail was presumably lost during library construction. The clone HFc3.47 contained 1713 nucleotides and overlapped HFc3.0 commencing in the ORF at nucleotide 583—the sequences of HFc3.0 and HFc3.47 were identical in the region of overlap. HFc3.47 also extended the 3′ UTR 937 nucleotides from the 3′ end of HFc3.0—a poly(A) tail and adenylylation signal were absent from this sequence and presumably were also lost during the cloning procedures. Clone HFc3.1 of 1444 nucleotides is almost entirely embodied within the sequence of HFc3.0 commencing at nucleotide 40 and extends 10 nucleotides beyond the adenylylation signal. This clone also contains a 117-nucleotide insertion at nucleotide 369 that introduces an in-phase termination codon into the ORF. Clone HFc3.1 may have been derived from a partially or aberrantly spliced mRNA molecule or may represent an mRNA splice product that would yield a smaller protein product. At this time it is not clear which of these possibilities is correct.

The ORF encodes a mature protein of 281 amino acids synthesized with a 34-amino acid leader sequence. It should be noted that there are two possible ATG initiation codons encoding methionine residues at position −34 and −30 (Fig. 1*b*). The first was predicted to be the start codon as it was the most 5′ ATG and adjacent sequences compared more favorably with the consensus sequences for initiation of protein synthesis (19). It is noteworthy that mouse FcγR cDNAs were also shown to contain multiple potential initiation codons (7–9). The N-terminal amino acid of the mature polypeptide chain was assigned by two criteria. First, the N-terminal sequence and leader sequence of the mouse α FcγR (7) were very similar to the corresponding regions in the human FcγR (Fig. 2) and were therefore used to predict the signal peptidase cleavage site. Second, the site chosen was in agreement with the consensus sequence of such sites based on the predictive system of von Heijne (20).

The mature human FcγR protein of 281 amino acids can be divided into a number of regions. The extracellular domain is comprised of 177 amino acids and contains several structural features. Like the mouse FcγR, there are four regularly spaced cysteine residues, suggesting that the human FcγR is also organized into two disulfide-bonded domains (Fig. 1*b*). The relationship of the two domains to each other was assessed by the Dayhoff algorithm and appropriate parameters (15, 21). Comparison of amino acids 7–85 (domain 1) to amino acids 87–168 (domain 2) shows 22/77 amino acid identities and a highly significant ALIGN score of 7.4 SD. This implies that the two domains may have arisen by tandem duplication of a single domain. One N-linked glycosylation site is present in each of the extracellular domains at Asn-61 and Asn-142. By contrast, the mouse α and β1 FcγRs have four authentic N-linked carbohydrate addition sites (22). The extracellular domain is followed by a 28-amino acid transmembrane sequence, predicted by a Kyte and Doolittle hydrophobicity plot (23) extending from residue 178 to the hydrophilic stop transfer sequence Arg^{206}-Lys^{207}-Lys^{208}-Arg^{209} (Fig. 1*b*). Following this membrane-spanning seg-

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          --->L                              +1      --->D1
MOUSE α       MFQNAHSGSQWLLPPLTILLLFAFADRQSAA--LPKAVVKLDPPWIQVLKEDMVTLMCEGTHNPG
              * **       *** *** *** * ** * **   **** ** **** ** ** *** * *   *
HUMAN         METQMSQNVCPRNLWLLQPLTVLLLLASADSQAAA--PPKAVLKLEPPWINVLQEDSVTLTCQGARSPE
                                 ** *     *     **** ******* ** ** ***** *   *
MOUSE β1       MESNWTVHVFSRTLCHMLLWTAVLNLAAGTHDLPKAVVKLEPPWIQVLKEDTVTLTCEGTHNPG
                                                        D1<---   --->D2
MOUSE α       NSSTQWFHNGRSIRSQVQASYTFKATVNDSGEYRCQMEQTRLSDPVDLGVISDWLLLQTPQRVFLEGET
                * ******  *    * ** ***  ****** **  ** ***** * * * ** ****   * ****
HUMAN         SDSIQWFHNGNLIPTHTQPSYRFKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGET
                * ******  *    * ** ***  ****** **  ** ***** * * * ** **** * * ****
MOUSE β1      NSSTQWFHNGRSIRSQVQASYTFKATVNDSGEYRCQMEQTRLSDPVDLGVISDWLLLQTPQLVFLEGET
                                                                    D2<---
MOUSE α       ITLRCHSWRNKLLNRISFFHNEKSVRYHHYKSNFSIPKANHSHSGDYYCKGSLGSTQHQSKPVTITVQD
              * ******  * *    ** * **         **** ********* * *  * *   *********
HUMAN         IMLRCHSWKDKPLVKVTFFQNGKSQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQV
              * ******  * *    ** * **         **** ********* * *  * **  *********
MOUSE β1      ITLRCHSWRNKLLNRISFFHNEKSVRYHHYSSNFSIPKANHSHSGDYYCKGSLGRTLHQSKPVTITVQG
                    --->Tm                          --->IC
MOUSE α       PATTSSISLVWYHTAFSLVMCLLFAVDTGLYFYVRRNLQTPREYWRKSLSIRKHQAPQDK
              *   **                          * *                        *
HUMAN         PSMGSSSPMGIIVAVVIATAVAAIVAAVVALIYCRKKRISANSTDPVKAAQFEPPGRQMIAIRKRQLEE
              *    * *   *** *   ******  * * *   **   *    *      *         *
MOUSE β1      PKSSRSLPVLTIVAAVTGIAVAAIVIILVSLVYLKKKQVPALPGNPDHREMGETLPEEVGEYRQPSGGS
HUMAN         TNNDYETADGGYMTLNPRAPTDDDKNIYLTLPPNDHVNSNN
                        *   *        *      *   *    *
MOUSE β1      VPVSPGPPSGLEPTSSSPYNPPKLEEAPKTEAENTITYSLLKHPEALDEETEHDYQNHI
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FIG. 2. Alignment of the human FcγR amino acid sequence with mouse α and β1 FcγR sequences (7, 9). Breaks (indicated by dashes) in the sequence have been introduced to optimize the alignment. Asterisks indicate identity between sequences. The leader sequence (L), extracellular domain 1 (D1), extracellular domain 2 (D2), transmembrane segment (Tm), and intracellular domain (IC) are shown. The amino-terminal residue is indicated by +1. The standard one-letter amino acid abbreviations are used.

ment, an in-frame termination codon is found at nucleotide 957, generating a 76-amino acid intracellular domain, which is mainly composed of polar and highly charged amino acids.

The primary sequence of the human FcγR predicts a protein with a molecular weight of 30,969, which may be glycosylated at the two N-linked glycosylation sites described and possibly O-linked sites as well.

Comparison of Human and Mouse FcγRs. The complete amino acid sequence of the human FcγR, aligned with the sequences of mouse β1 and α FcγRs, is shown (Fig. 2) with breaks introduced to optimize the alignment. The leader sequence of the human FcγR is very similar to the leader sequence of the mouse α FcγR with an ALIGN score of 7.7 SD (57% conservation of amino acids, 72% conservation of nucleotides) but has less similarity to the mouse β1 FcγR leader sequence (Table 1, Fig. 2). Comparison of the human FcγR domain 1 (Leu-7 to Ser-85) with the corresponding domain of the mouse β1 receptor gave 50/79 amino acid identities (ALIGN score, 23.4 SD) and ≈73% nucleotide similarity (Fig. 2, Table 1). Similarly, sequence comparison of domain 2 (Trp-87 to Thr-168) gave 51/82 amino acid identities (ALIGN score, 26.1 SD) and ≈74% nucleotide similarity between the human FcγR and mouse β1 FcγR (Fig. 2, Table 1). Similar identity was observed between the human FcγR and the mouse α FcγR (Fig. 2, Table 1) in the extracellular region. The two potential N-linked glycosylation sites are conserved between human and mouse FcγRs. While the leader sequence of the human FcγR is similar to that of the mouse α FcγR, the transmembrane sequence of the human FcγR is similar to the mouse β1 FcγR with 14/28 amino acid identities (ALIGN score, 3.4 SD) and 70% nucleotide similarity but shows little similarity to the transmembrane sequence of the mouse α FcγR (Fig. 2, Table 1). Comparison of the intracellular domain of the human FcγR with those of both mouse α and β FcγRs shows little identity of either nucleotides or amino acids (Fig. 2, Table 1). Finally, it was interesting to note that nucleotides 1797–1842 in the 3′ UTR showed considerable similarity (78%) to nucleotides 957–1002 in the coding sequence (the cytoplasmic tail) of murine β1 FcR (9). It would appear that by evolutionary processes this sequence has become redundant in the human gene and now represents vestigial "mouse" sequences.

Identity with Immunoglobulin Variable Domains. The structural features of the human FcγR protein classify it as a member of the immunoglobulin gene superfamily. Immunoglobulin domains are held together by disulfide bonds and fold in a characteristic pattern (β-strand structure) (24). Each of the β-strands of immunoglobulin domains have characteristic patterns of amino acid sequence and these can be identified in the FcγR sequence. The human FcγR contains four cysteine residues, which are probably involved in disulfide bonding to stabilize the formation of two domains. Both of these domains contain stretches of amino acids highly representative of the β-strands of immunoglobulin domains, including the sequence around the first cysteine in each domain, Val-Xaa-Leu-Xaa-Cys, which is characteristic of β-strand B, and the sequence around the second cysteine in each domain, Asp-Ser-Gly-Xaa-Tyr-Xaa-Cys, which is a characteristic immunoglobulin variable domain pattern and corresponds to β-strand F of immunoglobulin variable regions. Furthermore, a tryptophan residue located 13 residues downstream from the N-terminal cysteine in FcγR domain 1 is highly conserved among immunoglobulins and corresponds to β-strand C in immunoglobulin domains. All of these above features are conserved between the FcγRs of humans and mice and serve to classify these proteins as immunoglobulin gene superfamily members. In addition to the structural features noted, computer searches of protein data bases revealed significant similarity of domain 1 and, to a lesser extent, domain 2 with immunoglobulin κ-chain variable domains.

Table 1. Identity between human and mouse FcγR sequences

Human FcγR region	% identity with mouse α and β1 FcγRs			
	Amino acid		Nucleotide	
	α	β1	α	β1
Leader	57	14	72	31
Domain 1 (Leu-7–Ser-85)	61	63	70	73
Domain 2 (Trp-87–Thr-168)	60	62	74	74
Transmembrane	4	50	24	70
Intracellular	12	16	29	33

For definition of regions, see Fig. 2.

Expression of FcγR mRNA. RNA blot analysis demonstrated the presence of two mRNA transcripts of approximately 2.5 and 1.5 kb in normal spleen, which were absent from the FcγR$^-$ T-cell line MOLT-4 (Fig. 3). Furthermore, these transcripts were present in the myelomonocytic cell line THP-1, the erythroleukemia K562N, and the B-cell line Raji (data not shown). This pattern of expression of the two mRNA species correlates with the expression of FcγRII—the low affinity p40 FcγR. Furthermore, the expression of the FcγR mRNA in K562N cells excludes the possibility that either of the transcripts encodes FcγRI (the high-affinity p72 FcγR) or FcγR$_{lo}$ (low-affinity FcγR defined by the 3G8 mAb), as K562N cells are FcγRI$^-$, FcγR$_{lo}^-$ (2). The two mRNA transcripts found in these cells are likely to have been derived from the use of different polyadenylylation signals. The use of the adenylylation signal starting at nucleotide 1351 would yield a transcript of around 1.5 kb—the clones HFc3.0 and HFc3.1. The use of a second downstream adenylylation signal would give an mRNA of around 2.5 kb with an extended 3′ UTR, as is found with clone HFc3.47.

DISCUSSION

The results presented describe the molecular cloning of a human FcγR cDNA from a monocyte cDNA library by cross-species hybridization using the mouse β1 cDNA that encodes the low-affinity FcγRII and related synthetic sequences as probes. The similarity of both nucleotide and translated amino acid sequence of the cDNAs isolated with those of mouse FcγRs, together with RNA blot analyses, confirmed that the sequence described herein is indeed that

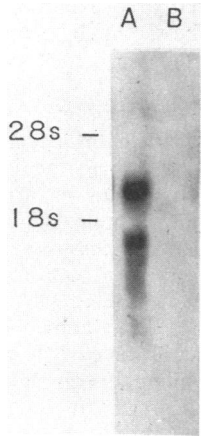

FIG. 3. Analysis of FcγR expression in normal human spleen by RNA blotting. Poly(A)$^+$ RNA from normal human spleen (lane A), and from the T-cell leukemia MOLT-4 (lane B), was probed with the cDNA insert from HFc3.1. The 28S and 18S ribosomal RNAs are indicated.

of a human FcγR. Based on the predicted amino acid sequence, the human FcγR is a typical transmembrane glycoprotein of 281 amino acids, which is synthesized with a hydrophobic leader sequence and contains a repeating extracellular domain, a transmembrane segment, and a relatively long intracellular domain.

The isolation of a human FcγR using the mouse FcγR cDNA as a probe was indicative of a substantial degree of similarity between the FcγRs of humans and mice. Several features of note were apparent when the sequences were compared. The amino acid similarity of the entire translated sequence was <50% (α = 49%, β = 47%), which is lower than has been described for other mouse and human cell-surface glycoproteins, including T200 (90%; ref. 25), CD5 (63%; ref. 26), CD4 (56%; ref. 27), and CD8 (56%; ref. 28). However, the degree of similarity varied from one region to another, the region of greatest similarity being the entire extracellular region (α = 60%, β = 62%) and the region of least similarity being the intracellular domain, which showed no similarity to the corresponding region of the α or β_1 mouse FcγR. Apart from the intracellular domain, the sequence of this human FcγR appears to be derived from sequences that are unique in the mouse α and β FcγRs. The human FcγR contains an α-like leader sequence, an α- and β-like extracellular domain, but a β-like transmembrane segment. The highly similar extracellular sequence may reflect this receptor's capacity to bind the same ligands as the mouse receptors; however, the significance of the chimeric nature of the human FcγR and its unique intracellular domain is not clear. The intracellular domain may correspond to an area of high evolutionary divergence, although it may also indicate that a different signal is imparted to the cell following ligand binding. It will be interesting to probe appropriate mouse mRNA with a sequence encoding the human intracellular domain to determine whether a similar gene can be found in the mouse. Similarly, to determine the possible diversity of human FcγR it will also be important to probe human mRNA with segments of the mouse FcγR sequences not found in the cDNA described herein, such as those encoding the β1 leader sequence, α transmembrane segment, and both α and β intracellular sequences to ascertain whether sequences similar to these are also present in humans. Indeed, Southern analysis (data not shown) showed the presence of multiple hybridizing restriction fragments indicating the possibility that, as in the mouse, other FcR genes remain to be characterized.

It was not surprising to find that the human FcγR possessed all the structural features of that in the mouse, including two conserved glycosylation sites and four conserved cysteine residues, which are probably involved in disulfide bonding to stabilize the formation of two domains as is found in the mouse (22). It was also noted that the two domains possessed a number of features characteristic of an immunoglobulin-related cell-surface glycoprotein. The observation that FcγRs belong to the immunoglobulin gene superfamily was first made by comparisons of mouse FcγR sequences with immunoglobulins (7–9). Characteristic invariant residues of immunoglobulin κ-chain variable regions are conserved in the FcγRs of both human and mouse.

The cloning of human FcγR cDNA will also enable the possible role of the FcγR in human disease states to be examined at the molecular level. It is known that increased levels of circulating immune complexes are a feature of several autoimmune disorders. It now remains to elucidate whether abnormal FcγR structure, expression, or regulation is responsible for the failure to clear immune complexes or can be implicated in the pathogenesis of autoimmune diseases, especially systemic lupus erythematosus, where altered FcγR function has been described (29).

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