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Cloning and sequence of the cDNA coding for rat type II Fcγ receptor of mast cells

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A clone encoding the rat type II Fcγ receptor was isolated from a cDNA library of the rat mucosal mast cell line RBL-2H3 and its sequence determined. The predicted amino acid sequence is highly homologous with the mouse type II as well as rat type III Fcγ receptors. The site of alternative splicing which generates the mouse FcγRb2 isoform is completely conserved. Hence we consider the new sequence to encode a rat FcγRII b2 isoform. In further analogy to the mouse receptor, no consensus motif known to be involved in accessory chain association was observed in the transmembranal domain. The importance of the identification of this receptor for investigation of the immunological stimulation of mast cells is discussed.

Fcγ receptor, RBL-2H3; Mast cell

1. INTRODUCTION

Receptors for the Fc domain of immunoglobulins (FcR) have been described for every Ig isotype. They provide an essential control and feedback link between the specific humoral and cell-mediated immune responses. This explains the fact that FcRs are found on all cell types of the immune system. In many cases several distinct Fc receptors are observed on one cell type.

The most widely distributed FcRs are those for the Fc domain of IgG (FcγR). The common structural feature of all Fc receptors (with one exception of FcεRII [1]) is an extracellular ligand binding unit containing two or three Ig-like domains [2]. Therefore they belong to the immunoglobulin superfamily [3]. Three main types of FcγR have been recognized in man and mouse [3]; FcγRI, FcγRII and FcγRIII. The type I Fcγ receptor binds monomeric IgG, types II and III, however, have lower affinity for IgG, hence they only bind its aggregates, i.e. immune complexes. Polymorphism in the ligand binding subunit of each type is considerable yet the main region of variability is the cytoplasmic tail. Altogether there are at least 12 different isoforms in human (for a review see [4]) with strictly controlled cell type expression. Studies aimed at resolving the signalling motifs of particular isoforms yield growing evidence for functional relevance of the extensive structural diversity. Only FcγRII has so far been found as a single chain receptor. The other FcγRs contain noncovalently associated accessory chains such as γ [5] or ζ [6] (originally identified in FcεRI and the CD3 complex, respectively) responsible, in the case of FcγRIII, for the signal transduction and membrane expression [7]. The rodent FcγRs system is clearly less complex than its human counterpart and therefore serves as a good experimental model in the effort towards understanding FcγRs structure – function relations. Rat Fcγ receptors have so far been less well characterized than those of the mouse. Rat FcγRIII has been cloned [8] and found, surprisingly, to be a family of homologous isoforms not encountered in the mouse [9]. The sequences of other rat FcγRs have not yet been determined.

The studies of the type I Fcε receptor, the principal receptor of mast cells and basophils, were pioneering and provided major advances in our understanding of the structure and function of Fc receptors. Thus rat mast cells, specifically those isolated from the peritoneum and the mucosal type line RBL-2H3, are those most widely employed as the experimental model systems for investigating the FcεRI structure and its coupling cascade to the mast cell function, e.g. the secretory response [10]. The capacity of RBL-2H3 cells to bind IgG class antibodies has been reported [11]. Further, a membrane component, distinct from FcεRI and FcεRII, binding rat IgE with low-affinity has been observed on rat mast cells (including RBL-2H3 cells) [12]. On human and mouse mast cells the presence of several isoforms of the low-affinity Fcγ receptors is known [13] and recent studies [14] showing their binding capacity also for IgE could rationalize the above result. However, the FcγRs on rat mast cells, particularly RBL-2H3 cells, have so far not been even structurally defined. Thus unequivocal determination of the types and struc-

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tures of rat mast cells Fcγ receptors is required. Here we report cloning of the rat FcγRII from RBL-2H3 cells. We provide evidence that in analogy to the mouse FcγRII, this clone is a b2 isoform of rat FcγRII.

2. MATERIALS AND METHODS

2.1. *Preparation of the probe by the polymerase chain reaction*

The probe for the rat cDNA library screening was prepared using the polymerase chain reaction on rat cDNA. The design of PCR primers was based on comparisons of cDNA sequences of mouse and human FcγRII in order to identify the most conserved stretches (Gen/EMBL: mouse m14216.em_ro, m31312.em_ro, m17515.em_ro and human m28696.em_pr, × 52473.em_pr, m31935.em_pr). Multiple sequence alignments were carried out using the University of Wisconsin GCG software package [15]. Two the most conserved regions showing, however, the least homology to the rat FcγRIII (Gen/EMBL m64370) were chosen for the synthesis of primers (forward primer = base pairs 104–123 and reverse primer = antisense to 714–733 in mouse m14216.em_ro). The PCR amplification was performed using random primed cDNA of RBL-2H3 cells (provided by Dr. M. Tal) and the conditions were as follows: 94°C, 1 min; 55°C, 2 min; and 72°C, 3 min in 34 cycles plus 10 min final extension at 72°C. The product of the reaction (approximate length 650 bp) was purified (DS-Primer Remover – Adv. Gen. Tech. Corp.), treated with Klenow enzyme (Boehringer) and subcloned into the *Sma*I site of a plasmid vector (BSII KS+/- Stratagene) for sequence analysis. Direct automatic dye-terminator sequencing (Applied Biosystems-AB 373A DNA sequencer) confirmed the expected high homology of the product (629 bp) to the mouse FcγRII. The subcloned product of the PCR amplification was excised from the plasmid and isolated after electrophoresis in low melting agarose. The insert was labeled (random primers labeling kit – Boehringer) with αdATP (Amersham) and used in the following library screening as well as Southern blotting.

2.2. *Rat cDNA library screening and DNA sequencing*

The rat cDNA library from RBL-2H3 cells was provided by Dr. M. Tal and M. Guthmann (Weizmann Institute). It was built using pcDNA I plasmid (Invitrogen, San Diego) in the MC1061/P3 *E. coli* strain Forty-eight pools each of 10^4 were subjected to PCR amplification with the same primers and conditions as used for probe preparation. Products were resolved on agarose gels and positive clones detected by Southern blotting (using Hybond N+ membranes-Amersham). Positive pools were screened by colony hybridization using stringent washing conditions (washing temp. 65°C, 0.2 SSC). Six positive clones were isolated and the length of inserts analyzed by agarose electrophoresis. The clone with the longest insert was selected and both DNA strands completely sequenced by direct automatic dye-terminator sequencing (Applied Biosystems -AB 373A DNA sequencer).

2.3. *Sequence analysis*

Analysis of the obtained DNA sequence was carried out using GCG software package [15]. DNA sequence was compared to the Gen/EMBL database and amino acids sequence to the NBRF and Swiss-Prot databases.

3. RESULTS

The probe for screening the rat cDNA library for FcγRII was prepared on the basis of a comparing cDNA sequences of known mouse and human FcγRII isoforms. The high homology among the extracellular domains (EC) of mouse and human type II and III FcγRs sequences is contrasted by marked differences in their transmembrane (TM) and cytoplasmic (IC) domains. We assumed this also holds for the rat. The probe was designed so as to contain stretches of the TM or IC regions in order to allow resolution between type II and known type III FcγRs. The PCR was used to amplify the sequence to be employed as a probe. Comparison of the human and mouse FcγRII cDNA sequences identified the most conserved stretches. We se-

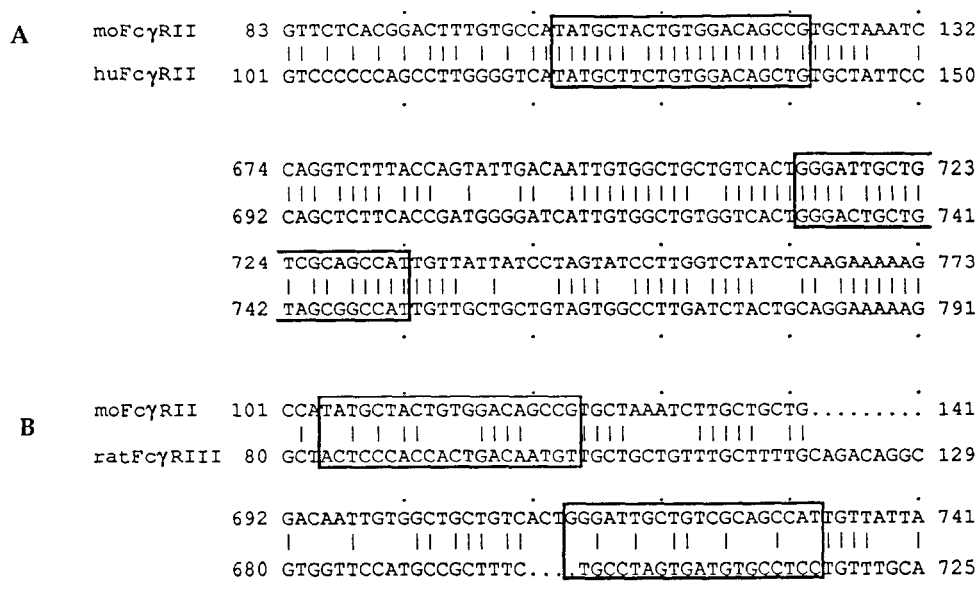
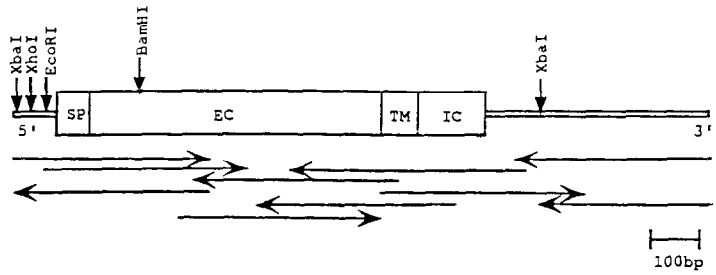


Fig. 1. Oligonucleotide sequences employed as PCR primers. In A, parts of the cDNA sequence of mouse FcγRII (Gen/EMBL m14216.em_ro) are aligned with homologous regions of the human FcγRII (Gen/EMBL m28696.em_pr). In B, the same regions of the mouse FcγRII sequence are aligned with the respective rat FcγRIII regions (Gen/EMBL m64370) Oligonucleotides synthesised as PCR primers are framed (forward primer – base pairs 104–123 and reverse primer – antisense to 714–733 in mouse FcγRII – Gen/EMBL m14216.em_ro).

a)



b)

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  1 CCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGGTGGAATTCTGCCAAACTCGGAGAGA 60
 61 AGCCTGTGCCTGCAGCTGACTCACTCCAGGACTGATGGGAACCCTGCTGTTCCTACCGCT 120
121 CCCC ATG GAC AGC AAC AGG ACT GTC GTC CAT GTG CTC TCA CGG ACT 166
-33      M   D   S   N   R   T   V   V   H   V   L   S   R   T  -20
167 TTG TAC CAT ATG CTA CTG TGG ACA GCT GTG CTA AAT CTT GTT GCT  211
-19 L   Y   H   M   L   L   W   T   A   V   L   N   L   V   A   -5
212 GAG TCT CAT GCA|GGT CTT CCG AAG GCT GTG GTG AAA CTT GAG CCC  256
 -4 E   S   H   A |G   L   P   K   A   V   V   K   L   E   P   11
257 CCA TGG ATC CAG GTG CTT AAG GAA GAC ACT GTG ACA CTG ATG TGC  301
 12 P   W   I   Q   V   L   K   E   D   T   V   T   L   M   C  26
302 GAA GGG ACC CAC AAC ACC AAG AAC TGT TCT ACT CAG TGG TTT CAC  346
 27 E   G   T   H   N   T   K  [N   C   S]  T   Q   W   F   H  41
347 AAT GGG AGC TCC ATC TGG CAT CAA GCC CAA GCC AAC TAC ACG TTT  391
 42[N   G   S]  S   I   W   H   Q   A   Q   A  [N   Y   T]  F  56
392 AAG GCC ACA GTC AAT GAT AGT GGA GAA TAC CGG TGC CGA ATG GAG  436
 57 K   A   T   V  [N   D   S]  G   E   Y   R   C   R   M   E  71
437 GAG ACC GGC ATC AGC GAA CCC ATA CAT CTG GGA GTG ATT TCT GAC  481
 72 E   T   G   I   S   E   P   I   H   L   G   V   I   S   D  86
482 TGG CTG CTA CTC CAG ACC TCT CAA CTG GTG TTT GAA GAA GGG GAA  526
 87 W   L   L   L   Q   T   S   Q   L   V   F   E   E   G   E  101
527 ACC ATC ACA TTA AGG TGC CAT AGC TGG AAG AAC AAA CAG CTG ACT  571
102 T   I   T   L   R   C   H   S   W   K   N   K   Q   L   T  116
572 AAG GTC TTG TTG TTC CAG AAT GGA AAA CCT GTG AGG TAT TAT CAT  616
117 K   V   L   L   F   Q   N   G   K   P   V   R   Y   Y   H  131
617 CAA AGC AGC AAT TTT TCA ATC CCC AAA GCC AAC CAC AGT CAC AGT  661
132 Q   S   S  [N   F   S]  I   P   K   A  [N   H   S]  H   S  146
662 GGG AAC TAC TAT TGC AAA GCA TAT CTA GGA AGG ACA ATG CAC GTG  706
147 G   N   Y   Y   C   K   A   Y   L   G   R   T   M   H   V  161
707 TCC AAG CCT GTC ACC ATC ACT GTC CAA GAG CCC AAA TCC AGC AGT  751
162 S   K   P   V   T   I   T   V   Q   E   P   K   S   S   S  176
752 TCG TTG CCG GTA TTG ACG ATT GTA GCT GCT GTC GCT GGA ATT GCT  796
177 S   L   P   V   L   T   I   V   A   A   V   A   G   I   A  191
797 GTA GCT GCC ATT GTT ATT ATC CTG GTA TCC TTG GTC TAT CTC AAG  841
192 V   A   A   I   V   I   I   L   V   S   L   V   Y   L   K  206
842 AAA AAG CAG GTT CCA GAC ACT CCT TCT GGT CTC GAA GAA GCT GAG  886
207 K   K   Q   V   P   D   T   P   S   G   L   E   E   A   E  221
887 AAA AAT GAG GTG GAG AAT ACA ATC ACC TAT TCA CTT CTC AAG CAT  931
222 K   N   E   V   E   N   T   I   T   Y   S   L   L   K   H  236
932 CCT GAA GCT CCG GAT GAA GAA TCA GAT CAC GAC TAC CAG AAC CAC  976
237 P   E   A   P   D   E   E   S   D   H   D   Y   Q   N   H  251
977 ATT TAG TCTCTCTTGAGATTGGGAAAAGCAAACCAGTAAGGCCAGGACCTAGCGTCTC 1034
252 I   *
1035 CTGGTCCAAGGAATGCTGTAGATATGAAAGAAAACATCTAGAGTCCCTTCTGTGAGTCCT 1094
1095 GAAACCAACAGACACTACGATATTGGTTCCCAATGGTTGACTGTACTAATGACTTCCATA 1154
1155 ACTTACAGCTTCCCAACTAAAGACTCTTTTGCTATAGATCCACACAGCCAATAAAATTAA 1214
1215 TCAACTTACTGTTGTTAAGAGACTACAGCAACGTGGGAACTGCTTATATTACATGCTAAG 1274
1275 GAGAACAAGTAACATTAGGATGCATAATCTCAGCGAGGATCAACTAGACGTGTACCAACC 1334
1335 TGTCAAAAGTGATGCTTCTTTATAAGAAACTTTATTTCCTTATTTTTCTATAAAGGTCAC 1394
1395 ATATTACTTTTATAGTAAAATGTTGTGAAAATGTCCAAAAAAAAAAAAAAAAAA      1448
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Fig. 2. Rat FcγRII. (a) Scheme of the cloned cDNA – open rectangles indicate coding region – signal peptide (SP), extracellular (EC), transmembranal (TM) and intracellular (IC) regions. Partial restriction map and the sequencing strategy is presented. (b) Nucleotide and translated amino acid sequences of the cloned cDNA. Translated sequence is presented in the one letter code. A signal peptide is predicted, cleavage site indicated by a bar between −1 and 1. *N*-Linked glycosylation sites are framed, cystein residues circled, predicted TM region overlined

lected those two which showed a low level of homology with rat FcγRIII. These sequences were then used to prepare the primers for PCR amplification (Fig. 1). Wherever the mouse and human DNA sequences differed, that of the mouse was used. The PCR amplification assay was performed using the above mentioned primers and randomly primed cDNA from RBL-2H3 cells as a template. Its product revealed a single band on agarose gel which matched well with the expected length of 629 bp (according to the mouse receptor sequence). The PCR product was then subcloned into the plasmid vector and sequenced. Comparison of the re-

sulting sequence with those present in the database showed its high homology to the mouse FcγRII (Gen/EMBL M14216) (85.2%) and the rat FcγRIII (Gen/EMBL M64368–70) (83.8%). However, the high homology with the extracellular domain of the latter one, contrasted with a markedly low homology in the transmembranal stretch, while the mouse FcγRII was, as expected, almost identical in this region. These results strongly supported the notion that the amplified sequence was that of the rat FcγRII and not the FcγRIII.

The probe was then used to screen the RBL-2H3 cDNA library. Six positive clones were isolated and that with the longest cDNA insert (1.5 kb) sequenced by the strategy depicted in Fig. 2a. Sequence analysis identified the only possible translational open reading frame (i.e. coding for a protein of the size close to mouse or human receptors). The sequence of the PCR product used as a probe was entirely contained within that reading frame. The 3′-noncoding region contains one polyadenylation signal (AATAAA) and a poly(A) tail of 18 nucleotides. The ORF of 855 nucleotides starts with initiation codon ATG at position 125, terminates at nucleotide 979, and encodes 285 amino acids. An amino-terminal signal sequence of 33 amino acids was predicted [16]. This leaves after signal peptide cleavage a protein of 252 amino acids (Fig. 2b). This final protein comprises an extracellular domain of 179 residues, a transmembrane domain of 26 and an intracellular one of 47 amino acids. The EC region contains six potential *N*-glycosylation sites [17] as well as four cysteine residues that most likely form the two disulfide bridges (Cys-26, -68, -107, -151) thus generating two Ig-like domains. This is in agreement with the principal structural features of other Fc receptors. A search of the SwissProt database showed highest homology with mouse FcγRII (percent identity 80.95) as well as with the rat FcγRIII (percent identity 67.96). However in the latter case the homology is limited only to the extracellular region. The TM stretch is, with the exception of single substitution of Thr for Ala, identical with the mouse homologous sequence. This confirms that FcγRII is single chain receptor and lacks sequences in its TM domain that lead to association with any known accessory chain. The IC domain has a 85.5% identity on the amino acid level with the respective mouse sequence. A search for possible consensus motifs in the IC domain, which could be endowed with signal capacity, revealed only 2 casein kinase II phosphorylation sites (residues 215, 244). The significance of this finding is presently unclear. As mentioned already, there are, in the mouse, two isoforms of FcγRII generated by alternative splicing of one exon (representing 47 amino acids) in IC tail. According to the size and full sequence conservation of the splicing site in rat receptor we consider the cloned receptor to be the rat analog of mouse FcγRII b2 isoform. Preliminary data exclude the presence of the b1 isoform in RBL-2H3 cells (Bocek P. et al., unpublished).

4. DISCUSSION

We have cloned and sequenced the cDNA encoding rat type II Fcγ receptor from the RBL-2H3 cell line. The sequence shows very high homology to its mouse counterpart (b2 isoform) as well as to the rat type III Fcγ receptor. Based on the complete conservation of the mouse splicing site sequence in the IC domain, we expect that a similar alternative splicing also occurs in the rat. High homology to the rat FcγRIII in EC domains is consistent with the patterns found in the mouse. Extensive homology between EC domains of the type II and type III Fcγ receptors in mouse (94% identity) has prevented, so far, preparation of antibodies which would differentiate between them. However, sequence homology of the EC domains of the cloned rat FcγRII and rat FcγRIII is rather lower (83.24% identity). This suggests the possibility of preparing of reagents with the above properties for studies in the rat system. This is of particular importance in the mast cell case where a substantial part of current research is done on rat cells. Recent experiments on mouse macrophages and B cells have shown that certain stretches of the cytoplasmic domains of different FcγRII isoforms are responsible for triggering of distinct functions in the respective cell lines [18]. Considering the high homology between rat and mouse FcγRII IC domains (85.5%) one can expect similar functions to be initiated via this receptor in the rat. The heteromeric FcγRIII or FcεRI use their accessory chains to initiate their signaling pathways. However, signaling cascades triggered by the distinct isoforms of FcγRII in different cell types are poorly understood. Studies in the cell types like mast cells (expressing FcRs for more than one isotype) have already been shown to be very informative [19,20]. We intend to investigate the functional role of the FcγRII in mast cells, particularly in relation to those initiated by the FcεRI.

The nucleotide sequence data reported in this paper will appear in the EMBL, DDBJ and GenBank Nucleotide Sequence Databases under the accession number X73371.

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