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Characterization and expression of an Fc_γ receptor cDNA cloned from rat natural killer cells

(IgG receptor/antibody-dependent cell-mediated cytotoxicity/rat CD16)

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ABSTRACT **A cDNA clone for an IgG-binding Fc receptor, rtFc$_\gamma$Rα, of the rat natural killer cell line CRNK-16 is characterized here. This clone encodes an Fc$_\gamma$ receptor as shown by the ability of cDNA-transfected COS cells to rosette IgG-coated sheep erythrocytes. The rtFc$_\gamma$Rα is exceptionally homologous to the mouse moFc$_\gamma$Rα, with 77% protein sequence identity and 71% nucleic acid identity overall. The transmembrane region of the rtFc$_\gamma$Rα contains the sequence Leu-Phe-Ala-Val-Asp-Thr-Gly-Leu, which is present in the membrane sequences of four other Fc receptors including mouse Fc$_\gamma$Rα, human Fc$_\gamma$RIII-2, and the Fc$_\epsilon$Rα subunits of the rat and human high-affinity IgE-binding receptors. Also, the rtFc$_\gamma$Rα cytoplasmic domain exhibits specific homology to other receptors derived from natural killer cells, human Fc$_\gamma$RIII-2 and mouse Fc$_\gamma$Rα. However, the rtFc$_\gamma$Rα cDNA clone is complementary to at least two different-sized mRNAs expressed by CRNK-16 cells, contrasting the single Fc$_\gamma$R-related mRNA species expressed by human and mouse natural killer cells. These rat mRNAs are homologous to both the 5′ and the 3′ end of the cDNA clone, suggesting that they may be (*i*) splice variants of one transcript or (*ii*) products of different but highly related genes.**

The antibody Fc receptors (FcRs) expressed on the surface of immune cells form an essential functional bridge between the humoral and cellular arms of the immune system. FcRs bind the Fc domains of antibodies in antibody–antigen complexes and trigger many cell-mediated immune processes such as phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC). The primary types of receptors involved in these immune processes are the IgG-binding FcRs (Fc$_\gamma$Rs) expressed on macrophages, lymphocytes, natural killer (NK) cells, granulocytes, and a variety of other cell types. Fc$_\gamma$Rs comprise a set of structurally diverse proteins mostly encoded by a family of homologous genes. Except for one structurally dissimilar form (1), Fc$_\gamma$Rs can be classified into three groups—Fc$_\gamma$RI, Fc$_\gamma$RII, and Fc$_\gamma$RIII—based on their protein sequences, ligand binding affinities, IgG subclass binding specificities, and/or cellular distributions (for reviews see refs. 2–4). Several cDNAs representing mouse and human Fc$_\gamma$RI high-affinity receptors (5, 6), mouse and human Fc$_\gamma$RII low-affinity receptors (7–10), and human Fc$_\gamma$RIII low-affinity receptors (11–13) have been described and multiple cell-type-specific isoforms have been identified for each Fc$_\gamma$R group. For example, at least three isoforms of mouse Fc$_\gamma$RII exist—moFc$_\gamma$Rα, moFc$_\gamma$Rβ1, and moFc$_\gamma$Rβ2—differing primarily in their transmembrane and cytoplasmic domain structures and in their expression by macrophages, lymphocytes, and other cell types (7, 14). Two isoforms of human Fc$_\gamma$RIII (CD16) have also been identified, Fc$_\gamma$RIII-2, a transmembrane protein expressed by NK cells, and Fc$_\gamma$RIII-1, a phosphatidylinositol-glycan-linked protein expressed by neutrophils (11, 13, 15–17).

The obvious complexity of Fc$_\gamma$R structures and their regulated expression by different cell types requires a broad-based approach for defining the essential features of these molecules. Interspecies comparisons of different Fc$_\gamma$R homologs have been highly informative in this regard. For example, both mouse and human Fc$_\gamma$RIs exhibit a third highly conserved extracellular domain (5, 6) in addition to two extracellular domains characteristic of Fc$_\gamma$RIIs and Fc$_\gamma$RIIIs, suggesting that this third domain contributes to the unique capacity of Fc$_\gamma$RIs to bind monomeric antibody. While mouse and human Fc$_\gamma$RII homologs have also been identified, species homologs of the human NK and neutrophil Fc$_\gamma$RIII (CD16) receptors have not been clearly defined. In this study, a cDNA cloned from rat NK cells is found to encode an Fc$_\gamma$R, designated rtFc$_\gamma$Rα or rat CD16,‡ that is exceptionally homologous to the mouse moFc$_\gamma$Rα. This receptor appears to be the functional species homolog of human huFc$_\gamma$RIII-2 and probably mouse moFc$_\gamma$Rα, originally identified in mouse macrophages (7) but recently shown by Northern hybridization to be homologous to the Fc$_\gamma$R expressed by mouse NK cells (14).

MATERIALS AND METHODS

Cell Lines. The following cell lines were used in this study: CRNK-16 cells, an interleukin 2-independent rat line adapted to culture (18) from a transplantable LGL (large granular lymphocyte) leukemia of Fischer 344 rats (19), generously provided by C. Reynolds (National Cancer Institute, Frederick, MD); COS cells, a simian virus 40 (SV40)-transformed monkey line (20), generously provided by C. Samuel (University of California, Santa Barbara); and J774 cells, a mouse monocytic line (21), obtained from the American Type Culture Collection. Cells were cultured in RPMI 1640 supplemented with 10 mM Hepes, 23 mM $NaHCO_3$, 4.6 mM reduced glutathione, 0.06 mM 2-mercaptoethanol, 2 mM glutamine, 50 units of penicillin per ml, 50 μg of streptomycin sulfate per ml, and 10% heat-inactivated fetal bovine serum. For CRNK-16 cells, this medium was also supplemented with 1 mM sodium pyruvate and 1× nonessential amino acids (Irvine Scientific).

Library Screening. A rat NK-cell λgt11 library made from CRNK-16 poly(A)$^+$ RNA was constructed by H. Young and C. C. Yue (National Cancer Institute, Frederick, MD) (22) and generously provided by C. Reynolds. Approximately 2 × 10^5 plaque-forming units were screened with full-length, random-primed (23) murine Fc$_\gamma$Rβ1 cDNA at 42°C in 5× SSPE/6% SDS/10× Denhardt's solution containing herring

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Abbreviations: FcR, Fc receptor; NK, natural killer; SRBC, sheep red blood cell; SV40, simian virus 40.
‡The sequence reported in this paper has been deposited in the EMBL/GenBank data base (accession no. M32062).

sperm DNA at 0.1 mg/ml. (SSPE is 0.18 M NaCl/0.01 M NaH_2PO_4, pH 7.4/1 mM EDTA; Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin.) Positive plaques were rescreened twice and one clone, λ6a6, was selected for further characterization.

Oligonucleotides. Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and, after purification, they were used either as DNA sequencing primers or as probes end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase (24).

Nucleotide Sequencing. Bacteriophage λ DNA was isolated from the λ6a6 clone by plate lysis (24) and the *Eco*RI-digested insert was subcloned into the sequencing phagemid pVZ-1, a derivative of pBluescribe (Stratagene) with an expanded polylinker site. Single-stranded DNA was isolated from the pVZ-λ6a6 construct (in both orientations) by using the M13K07 helper phage (Pharmacia) and was sequenced by the dideoxy chain-termination method (25) using Sequenase (United States Biochemical). DNA sequences were analyzed and compared to other known FcR sequences with the University of Wisconsin Genetics Computer Group (UWGCG) programs (26). The 1341-base cDNA sequence of rtFc$_\gamma$Rα was obtained from a total data base of 7189 sequenced nucleotides with an average of 5.36 independent sequencing gel determinations per nucleotide and with 90% of the cDNA sequence read from both strands.

Northern Blot Analysis. Total RNA was prepared from CRNK-16 and J774 cells by the guanidinium isothiocyanate method (24, 27). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose (Collaborative Research) chromatography (28), with slight modifications (29), and was electrophoresed in a 1% agarose/0.6 M formaldehyde gel and blotted to Hybond-N (Amersham) as described elsewhere (6). Filters were hybridized at 60°C with random-primed rtFc$_\gamma$R cDNA, or with a 700-base-pair (bp) *Nsi* I–*Eco*RI 3′ fragment of the cDNA (see Fig. 1), or with a 90-bp *Dra* I–*Eco*RI 3′ fragment (see Fig. 1). Hybridized filters were subsequently washed in 1× SSPE/0.5% SDS at 60°C and bands were visualized by autoradiography. Filters were stripped in boiling water between successive hybridizations.

COS Cell Transfections and Rosetting Assay. A 1341-bp *Eco*RI fragment containing the entire rtFc$_\gamma$Rα cDNA was subcloned by blunt-end ligation into the *Bam*HI site of the SV40 expression vector pJC119 (30) (kindly provided by C. Samuel). Two pJC-λ6a6 constructs, oriented in correct or incorrect orientations, were transfected into COS cells with DEAE-dextran (600 μg/ml; Pharmacia) (31) and chloroquine as previously described (32). Cells harvested 48 hr after transfection were tested for their ability to rosette IgG antibody-coated sheep red blood cells (SRBCs) (Sigma) prepared by preincubating a 10% (vol/vol) suspension of SRBCs for 30 min with rabbit anti-SRBC antiserum (Cappel Laboratories) at a 1:400 dilution. IgG-coated SRBCs were added to the transfectants in phosphate-buffered saline for 15 min at 37°C, and rosetted cells were visualized by light microscopy after staining with 0.5% crystal violet. RNA was also extracted from transfectants by lysis of the cells with 0.5% Nonidet P-40 and probed for rtFc$_\gamma$Rα-specific transcripts by dot blot analysis.

RESULTS

cDNA Isolation and Characterization. The rat CRNK-16 tumor cell line used for this study exhibits many of the phenotypic characteristics of normal rat NK cells (19). A rat λgt11 cDNA library constructed from CRNK-16 poly(A)$^+$ mRNA was screened with a full-length moFc$_\gamma$Rβ1 cDNA probe. One clone, λ6a6, containing a 1.3-kilobase (kb) *Eco*RI insert was fully characterized and shown to be an Fc$_\gamma$R-related sequence by Southern blot hybridization to an "Fc$_\gamma$R-specific" redundant oligonucleotide probe with the sequence 5′-CTGTCACCNCTYATRKCCACGGT-3′; this sequence is anticomplementary to coding sequences for a peptide, Asp-Ser-Gly-Glu-Tyr-Arg-Gln, found in most Fc$_\gamma$R proteins (6). The 1.3-kb insert was subcloned into the phagemid pVZ-1 and sequenced by the strategy outlined in Fig. 1.

The λ6a6 clone cDNA sequence, its predicted protein sequence, and the corresponding sequences of the mouse receptor moFc$_\gamma$Rα (7) are shown in Fig. 2. The rat-derived cDNA, designated rtFc$_\gamma$Rα, spans 1341 nucleotides with an open reading frame of 801 nucleotides specifying a 267-amino acid protein. An N-terminal 31-amino acid signal sequence (residues −31 to −1) is predicted (33), leaving a 236-amino acid protein after cleavage. The mature rtFc$_\gamma$Rα includes a 184-amino acid extracellular domain (residues 1–184), a 26-amino acid transmembrane domain (residues 185–210), and a 26-amino acid C-terminal cytoplasmic domain (residues 211–236). As indicated in Fig. 2, the extracellular domain contains five potential Asn-Xaa-Thr/Ser glycosylation sites and four cysteine residues that are most likely paired in immunoglobulinlike disulfide bonds (Cys-31 with Cys-73 and Cys-112 with Cys-156), on the basis of homology to other Fc$_\gamma$Rs and immunoglobulin superfamily protein structures (34, 35). Three additional cysteine residues exist in the transmembrane domain, but their potential disulfide-bonding status is uncertain. From the protein sequence, the calculated mass of rtFc$_\gamma$Rα is 26.7 kDa and, after glycosylation, the mass increases to ≈40 kDa if one assumes an additional 2.5 kDa per site glycosylated.

Comparison of Rat and Mouse Fc$_\gamma$Rα. The cDNA and protein sequences of rtFc$_\gamma$Rα and moFc$_\gamma$Rα are exceptionally homologous (Fig. 2). At the nucleotide level, 71% sequence identity is observed overall, with 75% identity in the coding regions and 43% identity in the 3′ untranslated regions after insertions and gaps are introduced to optimize homology. The two protein sequences are 77% identical overall with 76% identity in the extracellular domains, 80% identity in the transmembrane domains, and 68% identity in the cytoplasmic domains.

rtFc$_\gamma$Rα Expression. The full-length rtFc$_\gamma$Rα λ6a6 cDNA was subcloned into the SV40-based expression vector pJC119 and transfected into COS cells. Transient expression was assayed 48 hr later by testing the ability of transfected COS cells to rosette IgG-coated SRBCs. As shown in Fig. 3, COS cells transfected with rtFc$_\gamma$Rα cDNA in the correct orientation relative to the SV40 promoter rosetted antibody-coated SRBCs. However, mock-transfected COS cells or cells transfected with the cDNA in the inverted orientation failed to form rosettes (data not shown). RNA dot blots of cells transfected with rtFc$_\gamma$Rα in either orientation, but not of

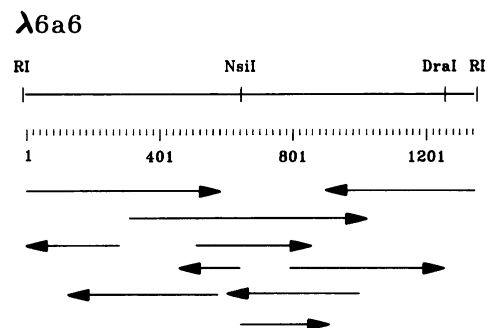

FIG. 1. Sequencing strategy and partial restriction map of the rtFc$_\gamma$Rα cDNA. The 1341-bp λ6a6 insert isolated from a λgt11 clone is represented in ruler-spaced increments of 20 bp. Arrows represent multiple sequencing runs performed with single- or double-stranded DNA. The *Eco*RI (RI) sites are derived from linkers used to construct the λgt11 cDNA library.

FIG. 2. Nucleic acid and predicted protein sequences of rat rtFc$_{\gamma}$Rα aligned with mouse moFc$_{\gamma}$Rα sequences. The protein and nucleic acid sequences of rtFc$_{\gamma}$Rα are shown on the top two lines and the corresponding moFc$_{\gamma}$Rα sequences are shown on the bottom two lines. Dashes signify identical bases in the two DNA sequences, periods signify gaps inserted in the DNA sequences to optimize alignment, and only amino acids unique to the moFc$_{\gamma}$Rα are shown. Asn-Xaa-Ser/Thr glycosylation-site motifs are marked by inverted triangles, with the glycosylation site at amino acid position 47 unique to the rat clone. Cysteine residues are boxed and a 26-amino acid transmembrane region is overlined. The boxed nucleic acid sequences (AATAAA) indicate polyadenylylation motifs.

mock-transfected cells, revealed high expression levels of rtFc$_{\gamma}$Rα RNA (data not shown).

As shown in Fig. 4*A*, Northern blot analysis of poly(A)$^{+}$ RNA isolated from CRNK-16 and J774 cells probed with the full-length rtFc$_{\gamma}$Rα cDNA clone revealed at least two CRNK-16 transcripts, 1.6 and 1.4 kb in size, and a broad cross-hybridizing J774 band; the width of the J774 band encompasses the range of sizes found for the three highly homologous Fc$_{\gamma}$R isoforms—moFc$_{\gamma}$Rβ1 (1.6 kb), moFc$_{\gamma}$Rβ2 (1.5 kb) and moFc$_{\gamma}$Rα (1.4 kb)—known to be expressed by this cell line (ref. 7 and D.W.S., unpublished data). A small (<0.2-kb), strongly hybridizing band of unknown origin also appeared in both lanes. The relationship between the 1.6- and 1.4-kb CRNK-16 transcripts was further investigated by reprobing the same filter with a 700-bp *Nsi* I-*Eco*RI fragment corresponding to the 3′ half of the rtFc$_{\gamma}$Rα cDNA (see Fig. 1) including the transmembrane, cytoplasmic, and 3′ untranslated sequences. As shown in Fig. 4*B*, this probe still hybridized to the two CRNK-16 transcripts but hybridized to a much narrower J774 band, matching the size of the moFc$_{\gamma}$Rα transcript as expected from the strong homology between rtFc$_{\gamma}$Rα and moFc$_{\gamma}$Rα in these regions. Because two polyadenylylation motifs were noted in the rtFc$_{\gamma}$Rα 3′ untranslated sequence (Fig. 2), this filter was reprobed with a 90-bp *Dra* I-*Eco*RI fragment (see Fig. 1) containing only the distal

FIG. 3. Rosetting assay with COS cells transfected with full-length rtFc$_{\gamma}$Rα cDNA. The rtFc$_{\gamma}$Rα cDNA was cloned into the SV40-based vector pJC119 and subsequently transfected into COS cells by the DEAE-dextran method. Transfected cells were added to rabbit anti-SRBC-coated sheep erythrocytes and positive rosettes were visualized by light microscopy. At upper-right center, a rtFc$_{\gamma}$Rα-transfected COS cell formed rosettes are shown by the surrounding clusters of antibody-coated SRBCs. At lower left, another COS cell did not rosette, presumably because it was untransfected in this experiment. Approximately 1% of the transfected cells formed rosettes in this experiment, where mock-transfected cells and cells transfected with the rtFc$_{\gamma}$Rα cDNA oriented incorrectly relative to the SV40 promoter failed to show any evidence of rosettes in parallel assays (data not shown). The results of this experiment are representative of several independent transfection experiments.

FIG 4. Northern blot analysis of poly(A)$^{+}$ RNA isolated from CRNK-16 and J774 cells. Six micrograms of poly(A)$^{+}$ RNA from J774 or CRNK-16 cells was run in each lane of a 1.0% agarose gel. The RNA was blotted onto a nylon filter and hybridized sequentially to full-length rtFc$_{\gamma}$Rα cDNA (*A*), a 708-bp *Nsi* I-*Eco*RI 3′ fragment (*B*), and a 92-bp *Dra* I-*Eco*RI 3′ fragment. The same Northern blot was used for each hybridization after stripping. Size markers at right are in kilobases.

polyadenylylation site (between nucleotides 1250 and 1341, Fig. 2) in order to determine whether these two transcripts result simply from differential polyadenylylation processing at these two sites. As shown in Fig. 4*C*, both CRNK-16 transcripts hybridized but no J774 bands were visible, consistent with the lack of homology between rtFc$_\gamma$Rα and moFc$_\gamma$Rα in this region (see Fig. 2).

DISCUSSION

A cDNA clone encoding a rat Fc$_\gamma$R, rtFc$_\gamma$Rα (rat CD16), has been isolated from rat NK cells and is characterized here. The 236-amino acid (≈40 kDa) glycoprotein encoded by this cDNA is proven to be an Fc$_\gamma$R by the acquired ability of transiently transfected COS cells to rosette IgG-coated SRBCs. The protein and DNA sequences bear striking homology to the mouse moFc$_\gamma$Rα protein (77% identity) and cDNA (71% identity) sequences (7), and these two receptors are the most homologous pair of Fc$_\gamma$R thus far isolated from separate species. The strong homology between these two receptors extends throughout their entire protein and DNA sequences including both the 5′ and the 3′ untranslated regions. However, slightly greater homology exists between the extracellular domains of rtFc$_\gamma$Rα and moFc$_\gamma$Rβ (see the Thr-Val-Thr-Leu-Thr sequence beginning at +26 in Fig. 2), but these two receptors are significantly less homologous overall, with only 55% protein and 56% nucleic acid sequence identity found between rtFc$_\gamma$Rα and moFc$_\gamma$Rβ1. These features of the rtFc$_\gamma$Rα extracellular domain have been confirmed by rat genomic clones having DNA sequences that closely match the extracellular domain sequence here except for 4 nucleotide and 4 amino acid differences that are suggestive of allomorphic variations arising from the different rat strain (Sprague–Dawley) used for the genomic cloning [Jos Even (Institut Curie, Paris), personal communication]. The exceptionally high level of extracellular-domain sequence homology between rtFc$_\gamma$Rα, moFc$_\gamma$Rα, and moFc$_\gamma$Rβ is also consistent with their antibody binding specificities for mouse IgG subclasses. In particular, cells expressing moFc$_\gamma$Rα or moFc$_\gamma$Rβ preferentially rosette cells coated with IgG1, -2b, or -2a antibodies but not IgG3 antibodies (7, 36). Likewise, in assays of antibody-dependent cell-mediated cytotoxicity, rat NK cells most efficiently lyse targets coated with IgG1, -2a, or -2b antibodies but less efficiently lyse IgG3-coated targets (E. S. Song, K. Young, and D.W.S., unpublished work).

As shown in Fig. 5, rtFc$_\gamma$Rα displays interesting homology to the transmembrane and cytoplasmic domains of four FcRs: moFc$_\gamma$Rα, human Fc$_\gamma$RIII-2 (11, 13), and the Fc$_\epsilon$Rα subunit of rat and human high-affinity IgE-binding receptors (37, 38). An 8-amino acid sequence, Leu-Phe-Ala-Val-Asp-Thr-Gly-Leu, is conserved in the transmembrane sequences of all five FcRs, and the aspartic residue in this sequence appears to be situated either directly within or bordering the membrane-spanning segment. The anomalous placement of a charged residue in a predominantly hydrophobic environment suggests the potential interaction of an accessory molecule such as the high-affinity IgE-receptor γ subunit, Fc$_\epsilon$Rγ, recently shown to form heterodimeric complexes with moFc$_\gamma$Rα (39).

The cytoplasmic sequence of rtFc$_\gamma$Rα also exhibits significant homology to moFc$_\gamma$Rα (73% identity) and to huFc$_\gamma$RIII-2 (34% identity) but little homology to the Fc$_\epsilon$Rα cytoplasmic sequence (Fig. 5). In particular, the C-terminal 4 amino acids, Pro-Gln-Asp-Lys, are conserved in the first three receptors. Because these are NK-specific FcRs, this evolutionarily conserved sequence, as well as the conserved transmembrane sequence discussed above, may be directly involved in the signal-transducing mechanism that triggers antibody-dependent NK-cell-mediated cytotoxicity.

The rtFc$_\gamma$Rα cDNA is unusual in that it identifies by Northern analysis at least two different transcripts in CRNK-16 cells, 1.4 and 1.6 kb in size; in contrast, mouse (14) and human (11) NK cells express transcripts of only one size. By densitometric scanning, these transcripts are found at equimolar levels and together they account for approximately one-fourth the level of homologous transcripts found in mouse J774 macrophages. The rat NK transcripts could not be differentiated by three rtFc$_\gamma$Rα probes matching the following portions of the cDNA: (*i*) its 3′ half, comprising the transmembrane, cytoplasmic, and 3′ untranslated sequences (Fig. 4*B*); (*ii*) its 5′ half, comprising the 5′ untranslated and extracellular sequences (data not shown); and (*iii*) its 3′-most 92-bp sequence, including the distal but not the proximal polyadenylylation motif (see Fig. 2), thereby ruling out the possibility that these mRNA species arise from alternative polyadenylylation of the same transcript (Fig. 4*C*). Overall, these transcripts exhibit homology to both the 5′ and the 3′ half of the rtFc$_\gamma$Rα cDNA, suggesting that at least two related rtFc$_\gamma$Rα isoforms are expressed by CRNK-16 cells. In fact, we have recently identified at least three distinct rtFc$_\gamma$Rα-related mRNA species amplified from CRNK-16 poly(A)$^+$ mRNA by the DNA polymerase chain reaction. Clones corresponding to these transcripts should establish whether they arise from alternative splicing, such as occurs with the moFc$_\gamma$Rβ1 and moFc$_\gamma$Rβ2 isoforms (7); whether they derive from different but highly homologous genes, such as occurs with transmembrane huFc$_\gamma$RIII-2 and phosphatidylinositol-glycan-linked huFc$_\gamma$RIII-1 isoforms (10, 16); or whether they represent yet another pattern of isoform variation unique to rat NK cells.

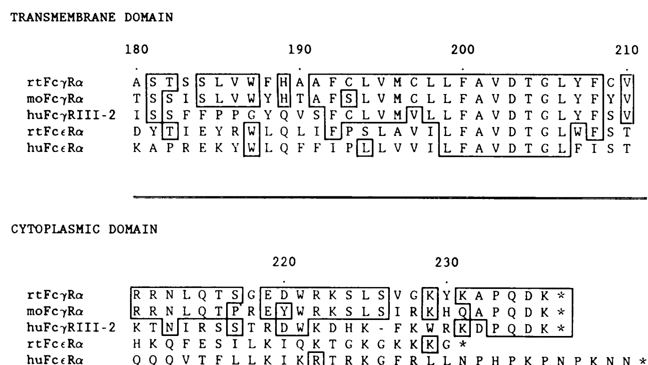

FIG. 5. Amino acid comparison of the transmembrane and cytoplasmic domains of rat rtFc$_\gamma$Rα, mouse moFc$_\gamma$Rα, human huFc$_\gamma$RIII-2, rat rtFc$_\epsilon$RI, and human huFc$_\epsilon$RI. Residues identical with the corresponding residues of the rtFc$_\gamma$Rα sequence are boxed.

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1. Simister, N. E. & Mostov, K. E. (1989) *Nature (London)* **337**, 184–187.
2. Unkeless, J. C., Scigliano, E. & Freedman, V. H. (1988) *Annu. Rev. Immunol.* **6**, 251–281.
3. Kinet, J.-P. (1989) *Cell* **57**, 351–354.
4. Fanger, M. W., Shen, L., Graziano, R. F. & Guyre, P. M. (1989) *Immunol. Today* **10**, 92–99.
5. Allen, J. M. & Seed, B. (1989) *Science* **243**, 378–381.
6. Sears, D. W., Osman, N., Tate, B., McKenzie, I. F. C. & Hogarth, P. M. (1990) *J. Immunol.* **144**, 371–378.
7. Ravetch, J. V., Luster, A. D., Weinshank, R., Kochan, J., Pavlovec, A., Portnoy, D. A., Hulmes, J., Pan, Y.-C. E. & Unkeless, J. C. (1986) *Science* **234**, 718–725.
8. Hogarth, P. M., Hibbs, M. L., Bonadonna, L., Scott, B. M., Witort, E., Pietersz, G. A. & McKenzie, I. F. C. (1987) *Immunogenetics* **26**, 161–168.

9. Lewis, V. A., Koch, T., Plutner, H. & Mellman, I. (1986) *Nature (London)* **324**, 372–375.
10. Brooks, D. G., Qiu, W. Q., Luster, A. D. & Ravetch, J. V. (1989) *J. Exp. Med.* **170**, 1369–1385.
11. Simmons, D. & Seed, B. (1988) *Nature (London)* **333**, 568–570.
12. Peltz, G. A., Grundy, H. O., Lebo, R. V., Yssel, H., Barsh, G. S. & Moore, K. W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1013–1017.
13. Scallon, B. J., Scigliano, E., Freedman, V. H., Miedel, M. C., Pan, Y.-C. E., Unkeless, J. C. & Kochan, J. P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5079–5083.
14. Perussia, B., Tutt, M. M., Qiu, W. Q., Kuziel, W. A., Tucker, P. W., Trinchieri, G., Bennet, M. & Ravetch, J. V. (1989) *J. Exp. Med.* **170**, 73–86.
15. Huizinga, T. W. J., van der Schoot, C. E., Jost, C., Klaassen, R., Kleijer, M., von dem Borne, A. E. G., Kr., Roos, D. & Tetteroo, P. A. T. (1988) *Nature (London)* **333**, 667–669.
16. Ravetch, J. V. & Perussia, B. (1989) *J. Exp. Med.* **170**, 481–497.
17. Ueda, E., Kinoshita, T., Nojima, J., Inoue, K. & Kitani, T. (1989) *J. Immunol.* **143**, 1274–1277.
18. Seaman, W. E., Eriksson, E., Dobrow, R. & Imboden, J. B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4239–4243.
19. Reynolds, C. W., Bere, E. W., Jr., & Ward, J. M. (1984) *J. Immunol.* **132**, 534–540.
20. Gluzman, Y. (1981) *Cell* **23**, 175–182.
21. Ralph, P., Prichard, J. & Cohn, M. (1975) *J. Immunol.* **114**, 898–905.
22. Ishikawa, H., Shinkai, Y.-I., Yagita, H., Yue, C. C., Henkart, P. A., Sawada, S., Young, H. A., Reynolds, C. W. & Okumura, K. (1989) *J. Immunol.* **143**, 3069–3073.
23. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
24. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
25. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
26. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
27. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
28. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
29. Clegg, D. O., Bodary, S. C., Large, T. H., Shelton, D. L. & Reichardt, L. F. (1989) in *Nerve Growth Factors*, ed. Rush., R. (Wiley, New York) pp. 255–275.
30. Sprague, J., Condra, J. H., Arnheiter, H. & Lazzarini, R. A. (1983) *J. Virol.* **45**, 773–781.
31. McCutchan, J. H. & Pagano, J. S. (1968) *J. Natl. Cancer Inst.* **41**, 351–356.
32. Luthman, H. & Magnusson, G. (1983) *Nucleic Acids Res.* **11**, 1295–1308.
33. von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4691.
34. Hibbs, M. L., Classon, B. J., Walker, I. D., McKenzie, I. F. C. & Hogarth, P. M. (1988) *J. Immunol.* **140**, 544–550.
35. Williams, A. F. & Barclay, A. N. (1988) *Annu. Rev. Immunol.* **6**, 381–405.
36. Weinshank, R. L., Luster, A. D. & Ravetch, J. V. (1988) *J. Exp. Med.* **167**, 1909–1925.
37. Kinet, J.-P., Metzger, H., Hakimi, J. & Kochan, J. (1987) *Biochemistry* **26**, 4605–4610.
38. Shimizu, A., Tepler, I., Benfey, P. N., Berenstein, E. H., Siraganian, R. P. & Leder, P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1907–1911.
39. Ra, C., Jouvin, M.-H. E., Blank, U. & Kinet, J.-P. (1989) *Nature (London)* **341**, 752–754.