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Immunology

Human FcγRIII: Cloning, expression, and identification of the chromosomal locus of two Fc receptors for IgG

(CD16/natural killer cells/phosphatidylinositol-linked protein)

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ABSTRACT **A cDNA clone encoding a human receptor for the Fc portion of IgG (FcγR), FcγRIII or CD16, was isolated from a human leukocyte library by a transient expression–immunoselection procedure. This cDNA (pGP5) encodes a 46-kDa phosphatidylinositol-linked cell surface protein with CD16 determinants and affinity for human IgG. The deduced protein sequence is most homologous to the murine receptor FcγRIIα, with slightly less homology to the human receptors FcγRII and FcεRI. The cDNA hybridizes to a 2.2 kilobase mRNA in human leukocytes and a cloned human natural killer cell line. FcγRIII is mapped to chromosome 1 by spot-blot analysis of sorted human chromosomes. Hybridization of FcγRII and FcγRIII probes to restriction digests of human genomic DNA separated by pulsed-field gel electrophoresis demonstrates physical linkage of the two genes within a maximum distance of 200 kilobases. The results identify a locus for at least two FcγR genes on human chromosome 1.**

Human leukocyte receptors for the Fc portion of IgG (FcγR) play important roles in immune responses by linking the humoral immune system with cellular effector functions. At least three distinct species of human FcγR have been distinguished on the basis of size, recognition by monoclonal antibodies, affinity for subclasses of human and murine IgG, distribution of expression on various cell types, and *in vitro* functionality (1). FcγRIII (CD16) is a 50- to 70-kDa membrane glycoprotein expressed by neutrophils, eosinophils, natural killer (NK) cells, and tissue macrophages that binds aggregated but not monomeric human IgG (2–5). That FcγRIII may be important in NK-cell function is suggested by the observations that the majority of NK activity in peripheral blood mononuclear cells (PBMCs) is contained within the subset of FcγRIII-expressing lymphocytes (6) and that this activity can be triggered through FcγRIII. The interaction of NK-cell FcγRIII with ligand or anti-FcγRIII antibodies results in cytokine production and expression of lymphocyte surface activation antigens (7, 8), induction of cytolytic activity (9 10), and inhibition of antibody-dependent cellular cytotoxicity (11). Neutrophil and tissue macrophage FcγRIII function in the clearance of antibody-coated erythrocytes or platelets (2, 5, 12) and in phagocytosis (13).

In this article we describe the identification and expression of a cDNA encoding human FcγRIII.¶ Cloned, expressed FcγRIII is a 46-kDa phosphatidylinositol (PI)-linked protein with significant sequence homology to other human Fc receptors. FcγRIII is mapped to chromosome 1 and physical linkage with human FcγRII is demonstrated.

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MATERIALS AND METHODS

Cell Lines and Antibodies. PBMC containing granulocytes were isolated from blood obtained from a normal donor by centrifugation over discontinuous density gradients of Ficoll/Hypaque. U-937, Daudi, RPMI 8866, and COS-7 cell lines have been described (14, 15). NL3, a cloned human $CD2^+3^-4^-8^-16^+$ NK cell line, will be described elsewhere. Culture supernatants containing monoclonal antibodies mAb32 and IV.3 were provided by C. Anderson (The Ohio State University, Columbus), B73.1 was from B. Perussia (Wistar Institute, Philadelphia), and 3G8 was from H. Fleit (State University of New York, Stony Brook). Leu11b, MOPC-21, and heat-aggregated human IgG have been described (14).

cDNA Clone Isolation and Characterization. A cDNA library of 2×10^6 independent clones was constructed using the pcD vector system (16) with the SRα promoter (17) from poly(A)$^+$ RNA isolated from neutrophil-containing human PBMCs. Transient expression of the cDNA library in COS-7 cells and immunoselection (panning) of transfected cells expressing FcγRIII using three anti-FcγRIII monoclonal antibodies (3G8, Leu11b, and B73.1) was performed as described (18), except that panning plates coated with $F(ab')_2$ fragments of goat anti-mouse IgG plus IgM (Jackson ImmunoResearch) were used. One of every four plasmids obtained after the third round of transfection and panning contained an identical 2.2-kilobase (kb) insert; one (pGP5) was selected for further analysis. COS-7 cells transiently expressing FcγRIII were visualized by indirect immunofluorescent staining as described (14). Restriction fragments of pGP5 were subcloned into m13mp18, m13mp19, and Bluescript vectors, and both strands of the entire insert were sequenced by the dideoxy method using modified bacteriophage T7 DNA polymerase (19). Computer-assisted DNA and protein sequence analysis was performed using the University of Wisconsin Genetics Computer Group programs (20).

RNA and DNA Blot Analysis. DNA was isolated from human peripheral blood leukocytes (21). RNA and DNA blot hybridizations were performed (14) using a ^{32}P-labeled 1.2-kb *Bgl* II–*Eco*RI restriction fragment of pGP5 as the FcγRIII probe. This fragment contains the entire coding region of FcγRIII except for the first 80 nucleotides. The FcγRII probe was prepared as two restriction fragments containing the entire FcγRII cDNA insert (14).

Abbreviations: FcγR, receptor for the Fc portion of IgG; PBMC, peripheral blood mononuclear cell; PFGE, pulsed-field gel electrophoresis; PI, phosphatidylinositol; PI-PLC, PI-specific phospholipase C; NK, natural killer.

¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04162).

Immunoprecipitation of FcγRIII. COS-7 cells transfected by electroporation with either FcγRII, FcγRIII, or no cDNA were cultured for 48 hr prior to surface iodination using lactoperoxidase. The radiolabeled cells were then subjected to digestion with 1.5 units of PI-specific phospholipase C (PI-PLC) from *Bacillus thuringiensis* (kindly provided by S. Udenfriend, Roche Institute of Molecular Biology) for 60 min (22). After centrifugation, immunoprecipitation of FcγRII and FcγRIII from the resulting supernatant and cellular fractions was performed (23) using monoclonal antibodies IV.3 and 3G8, respectively.

Chromosomal Mapping. Human chromosomes stained with two cDNA-specific dyes, Hoechst 33258 and chromomycin A_3, were identified and sorted by dual laser flow analysis. Approximately 3×10^4 human chromosomes of each type were sorted directly onto a nitrocellulose filter, denatured, and hybridized to ^{32}P-labeled *Bgl* II–*Eco*RI fragment of pGP5 as described (24, 25).

Pulsed-Field Gel Electrophoresis (PFGE). DNA was prepared from a human erythroleukemia cell line. A single-cell suspension (10^7 cells per ml) was embedded in blocks of 0.5% low-melting-temperature agarose (26). After equilibration with the appropriate digestion buffer, 40-μl blocks of agarose were incubated for 8 hr with 5–10 units of the indicated restriction enzymes. Digestion was terminated by washing the blocks in a solution of 50 mM EDTA and 50 mM Tris·HCl (pH 8), after which the blocks were melted at 70°C and gently pipetted into the wells of a 1% agarose gel. Fractionation was performed for 40 hr on a commercial apparatus (LKB Pulsaphor) at 330 V (10 V/cm) and 10°C with a switching time of 60 sec. The FcγRII and FcγRIII probes detected different bands in *Bst*NI-digested genomic DNA (data not shown), demonstrating that these two genes did not cross-hybridize under the conditions used.

RESULTS

Isolation of a cDNA Encoding FcγRIII. The FcγRIII cDNA clone pGP5 was isolated from a human leukocyte cDNA library in the pcDSRα expression vector by using the transient expression-immunoselection procedure of Seed and Aruffo (18). COS-7 cells transfected with this cDNA expressed functional FcγRIII protein that bound heat-aggregated human IgG and three anti-FcγRIII monoclonal antibodies (3G8, Leu11b, and B73.1). No staining of the transfected cells was observed with anti-FcγRII (IV.3) or anti-FcγRI (Mab32) monoclonal antibodies, nor with monomeric mouse IgG1, IgG2a, nor IgG2b at 0.1 mg/ml (data not shown).

RNA and Genomic DNA Blot Analysis. RNA blot analysis with the FcγRIII probe revealed hybridization to a 2.2-kb mRNA present in RNA isolated from a FcγRIII$^+$ human NK clone (NL3) and from human PBMCs (Fig. 1). mRNA from FcγRIII$^-$ human B-lymphoblastoid cell lines (RPMI 8866 and Daudi) and from a monocytic cell line (U-937) expressing FcγRI and FcγRII did not hybridize with the FcγRIII probe. In contrast to FcγRII (14), the FcγRIII probe hybridizes to restriction digests of genomic DNA from human PBMCs in a pattern suggesting a single-copy gene (Fig. 2).

cDNA Sequence Analysis. The complete ≈2-kb nucleotide sequence of the pGP5 cDNA insert and the predicted 233-amino acid protein sequence are presented in Fig. 3. A 699-base-pair open reading frame commences with a methionine codon at positions 18–20 and is followed by a 1.2-kb 3′ untranslated region. Analysis of the predicted amino acid sequence suggests an NH_2-terminal signal sequence of 18 amino acids (27). This region is followed by an ≈190-amino acid segment containing two tandem homology units exhibiting sequence homology to members of the immunoglobulin gene superfamily (28). Each of the repeating units has a pair of cysteine residues separated by 42–44 amino acids and two potential sites for N-linked glycosylation. The COOH-terminal region has a 20-amino acid hydrophobic segment containing a negatively charged aspartic acid residue, as is found in the transmembrane portions of murine FcγRIIα (29) and human FcεRI (30), followed by four hydrophilic amino acids. Comparison of the coding region of FcγRIII to other cloned Fc receptors reveals significant homology. FcγRIII is most homologous to murine FcγRIIα, with 62% nucleotide and 63% amino acid identity. FcγRIII also shares significant homology with murine FcγRIIβ$_2$ (29), human FcγRII (14), and human FcεRI, with 58%, 58%, and 53% amino acid identity, respectively.

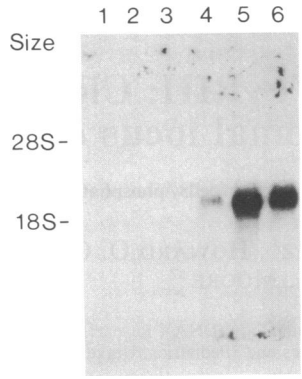

FIG. 1. RNA blot analysis of FcγRIII transcripts. Poly(A)-selected RNA (3 μg) from human lines RPMI 8866 (lane 1), Daudi (lane 2), U-937 (lane 3), NL3 (lane 6), and human PBMCs (lane 5) and 20 μg of total RNA isolated from human PBMCs (lane 4) were electrophoresed in a formaldehyde/agarose gel, blotted, and hybridized with a ^{32}P-labeled FcγRIII probe. Positions of the 28S and 18S RNAs are indicated.

While this manuscript was in preparation, a partial sequence of a human FcγRIII cDNA clone (31) was reported. Our sequence differs from that of Simmons and Seed (31) at four sites in the coding region (nucleotides 125, 210, 261, and 334). Differences at nucleotides 210 and 261 reduce the six potential N-linked glycosylation sites identified by Simmons and Seed (31) to four potential sites in our sequence, and the difference at nucleotide 261 generates a *Sal* I restriction site (positions 258–263) in our sequence, which is absent from their cDNA. The significance of these changes is uncertain, although at least two allelic forms of FcγRIII have been described (32). That these differences are not due to artifactual mutations arising from repeated passage of the pGP5 cDNA through COS-7 cells (33, 34) during the isolation procedure was established by analysis

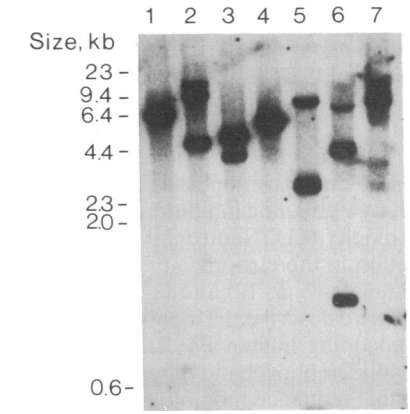

FIG. 2. Genomic DNA blot analysis. Aliquots (10 μg) of human PBMC DNA were digested with *Eco*RI (lane 1), *Bam*HI (lane 2), *Hin*dIII (lane 3), *Bgl* II (lane 4), *Pst* I (lane 5), *Nco* I (lane 6), and *Asp*718 (lane 7), electrophoresed in 1% agarose, blotted, and hybridized with a ^{32}P-labeled FcγRIII probe. Sizes of DNA standards are shown at the left.

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      10                  30                  50                  70  +1              90
CACTCCAGTGTGGCATCATGTGGCAGCTGCTCCTCCCAACTGCTCTGCTACTTCTAGTTTCAGCTGGCATGCGGACTGAAGATCTCCCAAAGGCTGTGGT
                MetTrpGlnLeuLeuLeuProThrAlaLeuLeuLeuLeuValSerAlaGlyMetArgThrGluAspLeuProLysAlaValVa
     110                 130                 150                 170                 190
GTTCCTGGAGCCTCAATGGTACAGGGTGCTCGAGAAGGACAGTGTGACTCTGAAGTGCCAGGGAGCCTACTCCCCTGAGGACAATTCCACACAGTGGTTT
lPheLeuGluProGlnTrpTyrArgValLeuGluLysAspSerValThrLeuLysCysGlnGlyAlaTyrSerProGluAspAsnSerThrGlnTrpPhe
     210                 230                 250                 270                 290
CACAATGAGAACCTCATCTCAAGCCAGGCCTCGAGCTACTTCATTGACGCTGCCACAGTCGACGACAGTGGAGAGTACAGGTGCCAGACAAACCTCTCCA
HisAsnGluAsnLeuIleSerSerGlnAlaSerSerTyrPheIleAspAlaAlaThrValAspAspSerGlyGluTyrArgCysGlnThrAsnLeuSerT
     310                 330                 350                 370                 390
CCCTCAGTGACCCGGTGCAGCTAGAAGTCCATGTCGGCTGGCTGTTGCTCCAGGCCCCTCGGTGGGTGTTCAAGGAGGAAGACCCTATTCACCTGAGGTG
hrLeuSerAspProValGlnLeuGluValHisValGlyTrpLeuLeuGlnAlaProArgTrpValPheLysGluGluAspProIleHisLeuArgCy
     410                 430                 450                 470                 490
TCACAGCTGGAAGAACACTGCTCTGCATAAGGTCACATATTTACAGAATGGCAAAGACAGGAAGTATTTTCATCATAATTCTGACTTCCACATTCCAAAA
sHisSerTrpLysAsnThrAlaLeuHisLysValThrTyrLeuGlnAsnGlyLysAspArgLysTyrPheHisHisAsnSerAspPheHisIleProLys
     510                 530                 550                 570                 590
GCCACACTCAAAGATAGCGGCTCCTACTTCTGCAGGGGGCTTGTTGGGAGTAAAAATGTGTCTTCAGAGACTGTGAACATCACCATCACTCAAGGTTTGG
AlaThrLeuLysAspSerGlySerTyrPheCysArgGlyLeuValGlySerLysAsnValSerSerGluThrValAsnIleThrIleThrGlnGlyLeuA
     610                 630                 650                 670                 690
CAGTGTCAACCATCTCATCATTCTCTCCACCTGGGTACCAAGTCTCTTTCTGCTTGGTGATGGTACTCCTTTTTGCAGTGGACACAGGACTATATTTCTC
laValSerThrIleSerSerPheSerProProGlyTyrGlnValSerPheCysLeuValMetValLeuLeuPheAlaValAspThrGlyLeuTyrPheSe
     710                 730                 750                 770                 790
TGTGAAGACAAACATTTGAAGCTCAACAAGAGACTGGAAGGACCATAAACTTAAATGGAGAAAGGACCCTCAAGACAAATGACCCCCATCCCATGGGAGT
rValLysThrAsnIleEnd

AATAAGAGCAGTGGCAGCAGCATCTCTGAACATTTCTCTGGATTTGCAACCCCATCATCCTCAGGCCTCTCTACAAGCAGCAGGAAACATAGAACTCAGA
GCCAGATCCTTTATCCAACTCTCGATTTTTCCTTGGTCTCCAGTGGAAGGGAAAAGCCCATGATCTTCAAGCAGGGAAGCCCCAGTGAGTAGCTGCATTC  1000
CTAGAAATTGAAGTTTCAGAGCTACACAAACACTTTTTCTGTCCCAACCATTCCCTCACAGTAAAACAACAATACAGGCTAGGGATGGTAATCCTTTAAA
CATACAAAAATTGCTCGTATTATAAATTACCCAGTTTAGAGGGGAAAAAAGAAAATAATTATTCCTAAACAAATGGATAAGTAGAATTAATGATTGAGGC  1200
AGGACCCTACAGAGTGTGGGAACTGCTGGGGATCTAGAGAATTCAGTGGGACCAATGAAAGCATGGCTGAGAAATAGCAGGGTAGTCCAGGATAGTCTAA
GGGAGGTGTTCCCATCTGAGCCCAGAGATAAGGGTGTCTTCCTAGAACATTAGCCGTAGTGGAATTAACAGGAAATCATGAGGGTGACGTAGAATTGAGT  1400
CTTCCAGGGGACTCTATCAGAACTGGACCATTTCCAAGTATATAACGATGAGCCCTCTAATGCTAGGAGTAGCAAATGGTCCTAGGAAGGGGACTGAGGA
TTGGGGTGGGGGTGGGGTGGAAAAGAAAGTACAGAACAAACCCTGTGTCACTGTCCCAAGTTAAGCTAAGTGAACAGAACTATCTCAGCATCAGAATGAG  1600
AATGAGAAAGCCTGAGAAGAAAGAACCAACCACAAGCACACAGGAAGGAAAGCGCAGGAGGTGAAAATGCTTTCTTGGCCAGGGTAGTAAGAATTAGAGG
TTAATGCAGGGACTGTAAAACCACCTTTTCTGCTTCAATGTCTAGTTCCTGTATAGCTTTGTTCATTGCATTTATTAAACAAATGTTGTATAACCAATAC  1800
TAAATGTACTACTGAGCTTCACTGAGTTACGCTGTGAAACTTTCAAATCCTTCTTCAGTCAGTTCCAATGAGGTGGGGATGGAGAAGACAATTGTTGCTT
ATGAAAAAAAGCTTTAGCTGTCTCCTGTTTTGTAAGCTTTCAGTGCAACATTTCTTGGTTCCAATAAAGCATTTTACA (A)n                  2000
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FIG. 3. Nucleotide sequence of pGP5 cDNA insert and predicted amino acid sequence of human FcγRIII. The +1, above the nucleotide sequence, indicates the start of the extracellular domain after the projected cleavage site of the secretory signal sequence. Four potential N-linked glycosylation sites are boxed. Cysteine residues that have the potential to form intrachain disulfide bonds are underlined. The hydrophobic amino acids at the COOH terminus are indicated with an overbar.

of a second FcγRIII cDNA isolated by hybridization with the pGP5 probe (pGP8; data not shown).

FcγRIII Has a PI Anchor. The sequence present at the COOH terminus of FcγRIII is similar to that of proteins known to be attached to the cell membrane by a PI anchor (35). To determine whether FcγRIII has a PI anchor, COS-7 cells expressing FcγRIII were labeled with ^{125}I, digested with PI-PLC, and centrifuged, and the resulting supernatant and cellular fractions were subjected to immunoprecipitation with the anti-FcγRIII monoclonal antibody 3G8 (Fig. 4). A substantial portion of surface-labeled FcγRIII was released from the cell membrane into the supernatant after PI-PLC digestion, indicating that FcγRIII has a PI anchor. PI-PLC digestion of transfected COS-7 cells expressing FcγRII (14) resulted in no detectable release of FcγRII from the cell membrane (Fig. 4).

FcγRII and FcγRIII Genes Are Closely Linked on Chromosome 1. Gene mapping of FcγRIII was accomplished by spot-blot analysis of sorted human chromosomes. FcγRIII hybridized to sorted human chromosome 1 (Fig. 5). Since the FcγRII gene(s) has also been mapped to human chromosome 1 (H.O.G., G.A.P., K.W.M., and R.V.L., unpublished data), we investigated whether these genes are adjacent to each other. FcγRII and FcγRIII probes were hybridized to human DNA digested with 10 "rare cutting" restriction enzymes and separated by PFGE. Both the FcγRII and FcγRIII probes detect identical fragments generated by the enzymes *Bss*HII, *Nae* I, *Nar* I, *Pvu* I, *Sal* I, and *Sst* II (Fig. 6). The sizes of these fragments range from 200 to 800 kb, demonstrating physical linkage of the two genes within a maximum distance of 200 kb. The FcγRII probe detects additional fragments (indicated by arrowheads) generated by the enzymes *Mlu* I, *Nru* I, and *Sfi* I that are not detected by the FcγRIII probe. This observation is

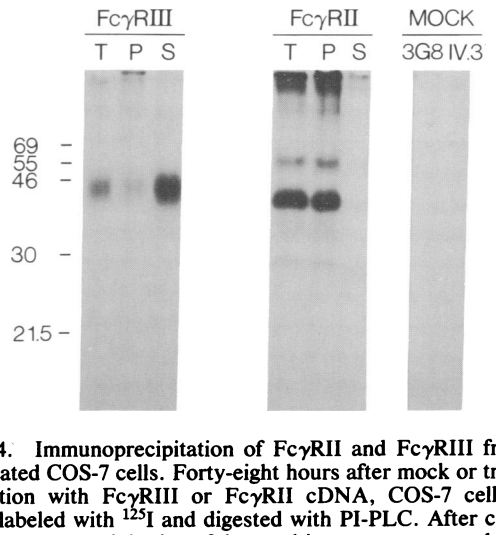

FIG. 4. Immunoprecipitation of FcγRII and FcγRIII from PI-PLC-treated COS-7 cells. Forty-eight hours after mock or transient transfection with FcγRIII or FcγRII cDNA, COS-7 cells were surface-labeled with ^{125}I and digested with PI-PLC. After centrifugation, immunoprecipitation of the resulting supernatant and cellular fractions was performed with anti-FcγRIII (3G8) or anti-FcγRII (IV.3) monoclonal antibodies. Immunoprecipitated proteins were analyzed by 12.5% $NaDodSO_4$/PAGE and autoradiography. Lanes show the proteins immunoprecipitated from the cell fraction of untreated cells (lanes T, 3G8, and IV.3), the cell fraction of PI-PLC-digested cells (lanes P), and the supernatant of PI-PLC-digested cells (lanes S). Relative molecular mass markers (in kDa) are indicated to the left.

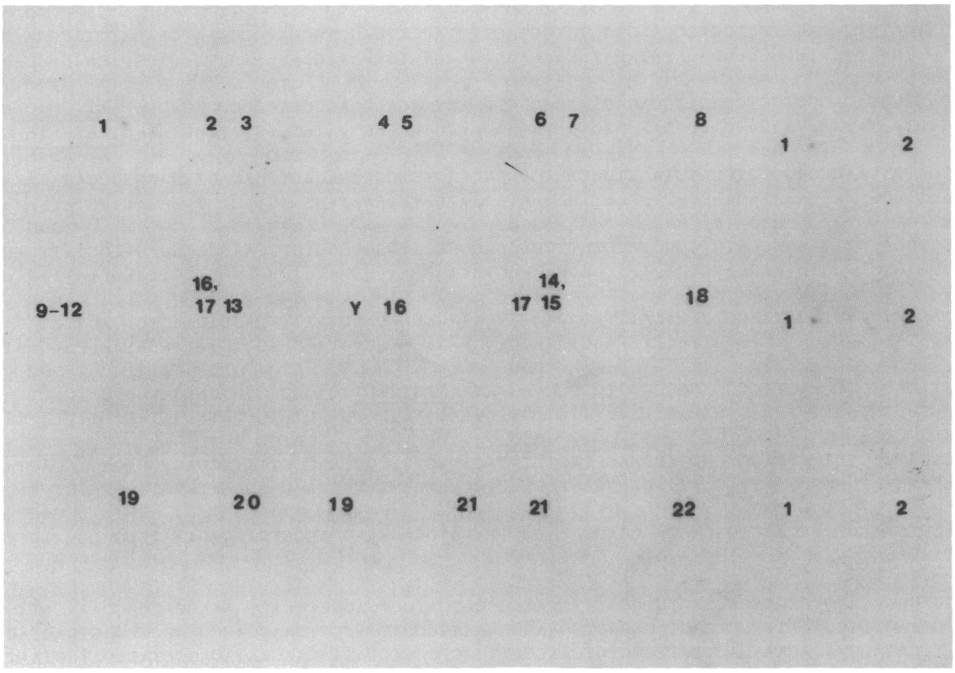

FIG. 5. Human FcγRIII mapped to chromosome 1. A spot-blot filter panel with circular filters each with two spots of sorted human chromosomes was hybridized to ^{32}P-labeled FcγRIII probe and autoradiographed. Chromosome numbers are indicated immediately adjacent to the locations of the sorted chromosomes. The chromosome 1 spot hybridized specifically to this probe. The spot on the chromosome 3,4 filter does not coincide with the location of the sorted chromosomes.

consistent with published data (14) showing that FcγRII hybridizes to multiple restriction fragments.

DISCUSSION

We have cloned and analyzed the molecular structure of the gene for human FcγRIII. Expression of the FcγRIII cDNA in COS-7 cells yields a 46-kDa PI-linked membrane protein with CD16 determinants and affinity for aggregated human IgG. The predicted amino acid sequence of FcγRIII exhibits two truncated immunoglobulin-like domains, each with a pair of cysteines separated by 42–44 amino acids, and has a high degree of homology with other cloned Fc receptors.

Data described here show that FcγRIII is a PI-linked membrane protein, as reported by others (31, 36, 37). Examination of the 3′ untranslated region of pGP5 does not clearly reveal sequences that might encode alternative transmembrane and cytoplasmic domains, as found in lymphocyte function-associated antigen 3 cDNAs (38, 39), although evidence for a PI-PLC-resistant FcγRIII on monocytes has been reported (37).

At present it is not understood how a PI-anchored membrane protein can mediate transduction of an activating signal to the cell, since it lacks a cytoplasmic domain that could interact with intracellular components. However, PI-linked proteins are known to exhibit a high degree of lateral mobility

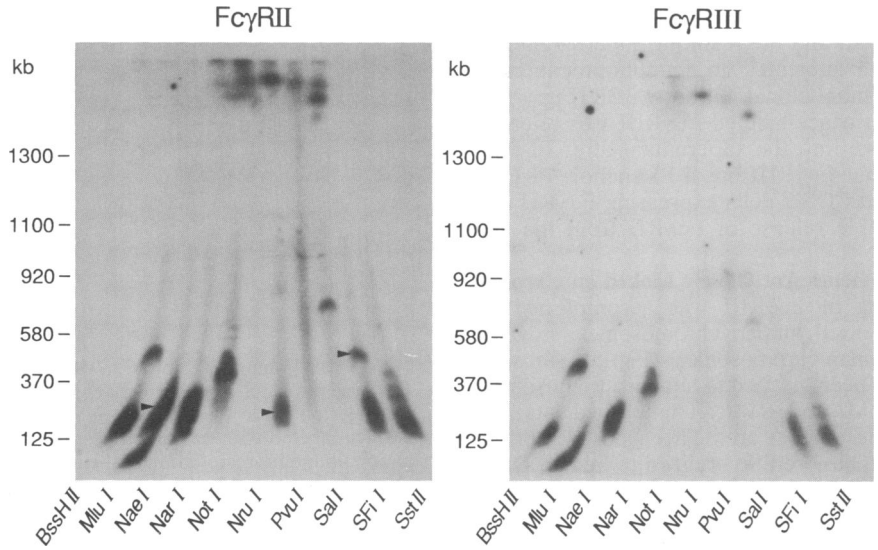

FIG. 6. Linkage of FcγRII and FcγRIII by PFGE and DNA blotting. DNA from a human erythroleukemia cell line was digested with the indicated restriction enzymes, fractionated by PFGE, blotted, and hybridized to ^{32}P-labeled FcγRII probe. The same filter was stripped of bound radiolabeled probe and hybridized to ^{32}P-labeled FcγRIII probe. Arrowheads indicate additional fragments hybridizing to the FcγRII probe that are not detected by the FcγRIII probe.

(35) in the cell membrane, which may facilitate their interaction with other signal-transducing cell surface molecules. For neutrophil FcγRIII, a possible candidate for interaction is FcγRII, which is a triggering molecule on these cells (37). Although FcγRII is probably not present in FcγRIII-bearing lymphocytes, FcγRIII might interact with other lymphocyte surface antigens, perhaps through heterophilic association (40) with those that also share an immunoglobulin-like structure. In this regard, we note reports of association of FcγR with class II major histocompatibility complex antigenic determinants (41). Soluble FcγRIII (42) released from human lymphocytes and neutrophils, possibly due to endogenous PI-PLC, may play a role in regulation of IgG synthesis (43) or in other immunoregulatory processes.

The finding that FcγRII and FcγRIII are closely linked on human chromosome 1 is of interest in several respects. These two genes may be members of a larger complex on chromosome 1 encoding other receptors with homologous sequences, analogous to the RCA locus (also on chromosome 1) encoding four receptors that bind to complement proteins C3b and C4b (44). The close linkage of the FcγRII and -III genes is likely to be relevant to the regulation of their expression on human hemopoietic cells. Finally, an association between certain HLA haplotypes and impaired FcγR-mediated immune complex clearance has been reported (45–47). FcγR-mediated phagocytosis by blood monocytes *in vitro* and IgG complex removal *in vivo* is significantly decreased in individuals with HLA haplotypes DR2 or DR3. Since the genes encoding FcγRII and FcγRIII are on human chromosome 1, they are not linked to the HLA genes on chromosome 6. Although the chromosomal localization of human FcγRI has yet to be determined, ligand binding by FcγRI apparently does not trigger endocytosis of monomeric human IgG (48). In view of these findings, it is unlikely that the association between HLA haplotype and FcγR-dependent immune complex clearance is due to physical linkage of their respective genes.

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