Expression Cloning of a Human Fc Receptor for IgA

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Summary

IgA, the predominant isotype in secretions, mediates the neutralization and removal of environmental antigens from mucosal sites. Although cell surface receptors for the Fc region of IgA (FcαR) have been implicated in a variety of immune effector mechanisms, the molecular features of FcαR remain only marginally characterized. In this report, we describe the isolation of a clone from a myeloid cell line cDNA library that directs the expression of a cell surface molecule with IgA binding specificity. The cDNA encodes a peptide of M_r 30,000 including a putative transmembrane region with features atypical of conventional membrane-anchored proteins. Databank searches indicate that the human myeloid cell FcαR sequence is unique, is a member of the immunoglobulin gene superfamily, and is related to Fc receptors for IgG (FcγRI, II, and III) and IgE (FcεRI).

IgA fulfills a critical protective role against the constant environmental insults encountered at mucosal surfaces (1). Although the mechanism of protection is not completely defined, the IgA-mediated mucosal immune response may be at least partially manifested through binding of IgA-coated targets to Fc receptors (FcαR) on effector cells. In particular, human monocytes, macrophages, neutrophils, and myeloid cell lines express cell surface FcαR (2–5) that have been shown to mediate effector functions such as phagocytosis (6–8), antibody-dependent cell cytotoxicity (9), and inflammatory mediator release (10, 11). Other studies indicate that the human myeloid cell FcαR is a protein of ~60 kD (12, 13), which, upon deglycosylation, can be resolved to two protein cores of 32 and 36 kD (13).

Recently, a mAb (My43) was developed that inhibited binding of IgA and phagocytosis of IgA-coated targets, and that could directly trigger phagocytosis and superoxide production by human myeloid cells and cell lines (14). Furthermore, the mAb failed to bind to human lymphocytes, consistent with the likelihood that My43 binds to a myeloid cell–specific IgA receptor. In this report, we describe the use of the My43 mAb to isolate a clone from a myeloid cell line cDNA library that directs the expression of a cell surface molecule with IgA binding specificity.

Materials and Methods

Cells. The monocyte-like cell line U937 (15) was maintained in continuous culture in RPMI 1640 supplemented with 10% FCS. Differentiation was induced by culturing the cells overnight in the presence of 5 ng/ml of PMA (Sigma Chemical Co., St. Louis, MO). Monocytes and polymorphonuclear leukocytes were purified from heparinized peripheral blood by centrifugation through isolymph (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY), followed by centrifugal elutriation, using a centrifuge (6 M/E; Beckman Instruments, Inc., Fullerton, CA) equipped with a JE-5.0 rotor. The resulting fractions were analyzed by Wright-Giemsa staining of cytocentrifuge preparations.

Human T lymphocytes were purified from tonsils by E-rosetting with 2-aminoethylisothio-uronium bromide (AET)[1]-treated SRBC, followed by Ficoll-Hypaque density gradient centrifugation (16). Tonsillar B lymphocytes were isolated from the leukocyte layer resulting from AET-SRBC and Ficoll separation, with further depletion of non-B cells achieved by treatment with antibody and complement using Lympho-Kwik B (One Lambda, Los Angeles, CA). Purified preparations of T and B cells were maintained in a medium consisting of RPMI 1640/10% FCS (HyClone Laboratories, Logan, UT), sodium pyruvate (1 mM), nonessential amino acids (100 mM), penicillin/streptomycin (100 U/ml per 100 μg/ml), L-glutamine (2 mM), 2-ME (50 mM), and fungizone (1:2,000; Gibco Laboratories, Grand Island, NY). B cells or T cells were cultured either alone or, as indicated, in the presence of Con A (20 μg/ml; Sigma Chemical Co.), PWM (40 μg/ml; Sigma Chemical Co.), or PMA (5 ng/ml) plus ionomycin (500 ng/ml; Calbiochem-Behring Corp., San Diego, CA). After a 16-h incubation, cells were harvested for RNA preparation as described below.

cDNA Expression and Immunoselection. cDNA was synthesized from polyadenylated RNA extracted from 12-h PMA-stimulated U937 cells according to standard protocols (17). The cDNA was

[1] *Abbreviations used in this paper:* AET, 2-aminoethylisothio-uronium bromide; GPI, glycosyl-phosphatidyl inositol; OE, ox erythrocyte; PLC, phospholipase C.

ligated into pDC303, a modified version of the pDC302 mammalian expression plasmid (18), using nonselfcomplementary linkers containing BglII restriction sites.

The cDNA library representing 600,000 independent clones was amplified by growing bacteria in liquid cultures in the presence of chloramphenicol overnight, then plating at a density of 5,000 colonies per plate. Colonies were scraped from plates and pooled, and plasmid DNA was recovered and purified by alkaline lysis and CsCl gradient centrifugation (19). Plasmid DNA from the cDNA library was used to transfect a subconfluent layer of monkey COS-7 cells using DEAE dextran followed by chloroquine treatment, as described (20). FcαR$^+$ cells were selected by incubation in the My43 anti-FcαR mAb (14), followed by panning on anti-IgM-coated plates according to established procedures (21). Episomal DNA was prepared from the panned cells, amplified, and reintroduced into COS cells. After three such rounds of transfection (two DEAE dextran rounds and one protoplast fusion round) and immunoselection, 96 individual plasmid-transformed bacterial colonies were grown, collected into 20 pools, and pooled plasmids were extracted by alkaline lysis (19) and transfected into COS cells. Positive pools were selected by flow cytometric analysis of anti-FcαR (My43) binding to transfected cells. Individual clones containing FcαR cDNA were isolated from positive pools. One of these, pHuFcαR, was selected for characterization.

Sequence Analysis. DNA sequencing was performed on both strands as previously described (22). For alignment analysis, residues constituting the two extracellular Ig domains were aligned with other FcR Ig domains using computerized models (NBRF program ALIGN[23]) and by eye. The MD data matrix was used with a bias of +6, and a gap penalty of 6 was used. Scores are the SD for the indicated pair of aligned sequences vs. the average scores from 100 randomized alignments of the same pair of sequences.

Immunoglobulins. The My43 anti-FcαR murine hybridoma cell line (IgM, κ) was maintained in Dulbecco's medium containing 10% FCS. Supernatants were collected at twice-weekly intervals, sterile filtered, and stored at 4°C. Human IgA1 and IgG1 paraproteins were purified as previously described (14). F(ab')$_2$ anti-ox erythrocytes (OE) was prepared from rabbit anti-OE IgG (Cooper-Biomedical, Inc., Malvern, PA) by pepsin cleavage and passage through protein A-Sepharose CL-6B.

Flow Cytometry. For anti-FcαR binding experiments, cells were incubated with hybridoma culture supernatants containing either My43 mAb or a nonspecific isotype control mAb. After 30 min at 4°C, cells were washed and incubated with fluoresceinated second antibody (affinity-purified goat anti-murine IgM, μ chain specific [F(ab')$_2$]) for 30 min at 4°C, then washed. For IgA binding experiments, cells were stained with 12.5 μg/ml FITC-conjugated human myeloma IgA1 or IgG1 (30 min at 4°C). All antibody dilutions and washes were performed in PBS/0.1% BSA/0.05% sodium azide. Cells were analyzed on a FACScan flow cytometer using a logarithmic fluorescence intensity scale.

Rosette Assay. Conjugates of IgA or IgG linked to anti-OE were made by using the bifunctional reagent SPDP (Pharmacia Fine Chemicals, Uppsala, Sweden) (24). IgA or IgG and F(ab')$_2$ anti-OE (at 1–3 mg/ml) were treated separately with an eightfold molar excess of SPDP for 2 h at 18°C. SPDP-treated anti-OE F(ab')$_2$ was dialyzed in PBS, pH 7.2, and SPDP-treated IgA and IgG were dialyzed in 0.1 M acetate, 0.1 M NaCl, pH 4.5, treated with 0.02 M dithiothreitol (30 min), and passed through a G-25 Sephadex column (Pharmacia Fine Chemicals), equilibrated in 0.1 M phosphate, 0.1 M NaCl, pH 7.5. Equimolar amounts of the anti-OE F(ab')$_2$ and IgA or IgG were then mixed and incubated at 18°C for 4 h, after which crosslinking was terminated with 2 mM iodoacetamide. Preparations contained <15% noncrosslinked Ig.

Packed OE (10 μl) were mixed for 16 h at 10°C with 25 μl of the F(ab')$_2$ conjugates at concentrations previously determined to give maximal rosette formation. Equal volumes of cells (transfected COS-7 or U937 cells) at 2 × 10^6/ml and a 1% suspension of heteroantibody-coated OE were centrifuged together and incubated on ice for 90 min. In some cases, cells were preincubated on ice with human IgA, IgG, My43, or control IgM mAb before the addition of OE. After gentle resuspension, the percentage of rosette-forming cells, defined as cells binding greater than four OE, was determined by counting a minimum of 200 cells in duplicate samples using light microscopy.

Hybridization Techniques. Total RNA was isolated from cells as described (25), then subjected to oligo(dT)-cellulose chromatography to purify polyadenylated RNA (19). The mRNA was subjected to electrophoresis in a formaldehyde/1.2% agarose gel, blotted by capillary flow onto a Nytran filter (Schleicher & Schuell, Inc., Keene, NH) (26), and hybridized for 16 h at 63°C in Starks hybridization solution (50% formamide, 2× Denhardt's solution, 5× SSC, 0.1% SDS, 50 mM KH_2PO_4, 20 mM EDTA, 0.05% *N*-lauroylsarcosine, 150 μg sheared, denatured salmon sperm DNA per ml) containing ^{32}P-labeled antisense riboprobe transcribed from the 5′ portion (~430 bases) of pHuFcαR. Washes were carried out in 2× SSC, 0.1% SDS at 63°C. Dried filters were exposed to Kodak X-Omat AR film for 2 d and developed.

Human placental genomic DNA was isolated as described (27) and digested to completion with the indicated restriction endonucleases. 8 μg of digested DNA was subjected to electrophoresis on a 0.7% agarose gel, transferred to a Nytran filter, and hybridized for 16 h at 42°C in Starks solution containing a ^{32}P-labeled 700-bp fragment encoding the 5′ untranslated and extracellular coding region of FcαR. Hybridized blots were washed in 0.2× SSC, 0.1% SDS at 65°C, dried, and autoradiographed.

Results and Discussion

The My43 mouse mAb binds selectively to a cell surface receptor for IgA on human myeloid cells. We used this mAb as the basis for immunoselection of an FcαR cDNA clone, using a modification of the technique previously described by Seed and Aruffo (21). The U937 cell line represented a convenient source of mRNA for cDNA library construction, since FcαR expression as measured by My43 binding is inducible by PMA treatment. We found that maximal FcαR cell surface expression occurred after 24 h in the presence of PMA, suggesting that maximal mRNA levels are achieved at an earlier time point. Thus, polyadenylated RNA was extracted from U937 cells treated for 12 h with PMA, and a cDNA library was prepared in the pDC303 mammalian expression plasmid.

Purified plasmid DNA from the U937 cDNA library was transfected into COS-7 cells, FcαR-expressing cells were isolated by panning with the My43 mAb, and plasmid DNA was extracted from the immunoselected COS-7 cells. After additional rounds of transfection and immunoselection, an individual FcαR cDNA clone was selected by flow cytometric analysis of transfected COS cells using the My43 mAb. As shown in Fig. 1 *A*, My43 bound to a subpopulation of COS cells transfected with pHuFcαR, but failed to react with cells transfected with an insertless plasmid. An isotype control mAb displayed only background binding with both cell types (Fig. 1 *B*). Besides expressing a cell surface epitope recognized by

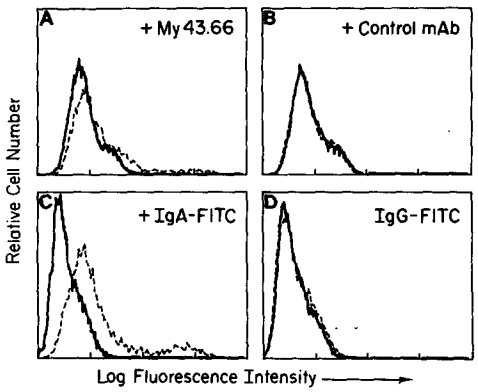


Figure 1. Expression of FcαR cDNA in transfected cells. (*A* and *B*) Anti-FcαR binding. Control plasmid (*solid line*) and pHuFcαR (*dashed line*) transfected COS cells were stained with either My43 or a murine IgM isotype control and analyzed by flow cytometry. (*C* and *D*) IgA binding. Control plasmid and pHuFcαR transfected COS cells were stained with human IgA-FITC or human IgG-FITC and analyzed by flow cytometry.

My43, these cells were also capable of binding IgA. Thus, FcαR-transfected COS cells directly bound FITC-conjugated human IgA (Fig. 1 *C*), but not human IgG-FITC (Fig. 1 *D*). In contrast, control COS cells displayed only background binding of either Ig isotype. The receptor exhibited the expected binding specificity, since IgA-FITC binding was inhibited by unconjugated human IgA and by My43, but not by human IgG or an irrelevant murine IgM mAb (not shown).

Table 1. *Inhibition of FcαR⁺-COS Cell IgA Rosette Formation*

Antibody treatment	Concentration	Rosette-forming cells	
		%	*SD*
Medium	–	10.6	(0.9)
My43 hybridoma supernatant	Undiluted	1.5	(1)
	1:3	0.75	(0.75)
	1:9	8.3	(0.25)
	1:27	11.5	(0.15)
Control hybridoma supernatant	Undiluted	11.4	(1)
Human IgA	2 mg/ml	0	
	666 μg/ml	0.9	
	222 μg/ml	6.2	(0.3)
	74 μg/ml	9.3	(1)
Human IgG	2 mg/ml	9.7	(0.05)

FcR⁺ COS cells were treated for 30 min at 4°C with hybridoma supernatants or purified Ig, or with medium alone. The cells were then examined for ability to form rosettes with OE coated with human myeloma IgA, as described in Materials and Methods. Results show mean and SD of duplicates.

FcαR expression was also measured by the ability of transfected cells to form rosettes with IgA-coated OE (Fig. 2). COS cells transfected with pHuFcαR formed distinct rosettes when incubated in the presence of human IgA-coated

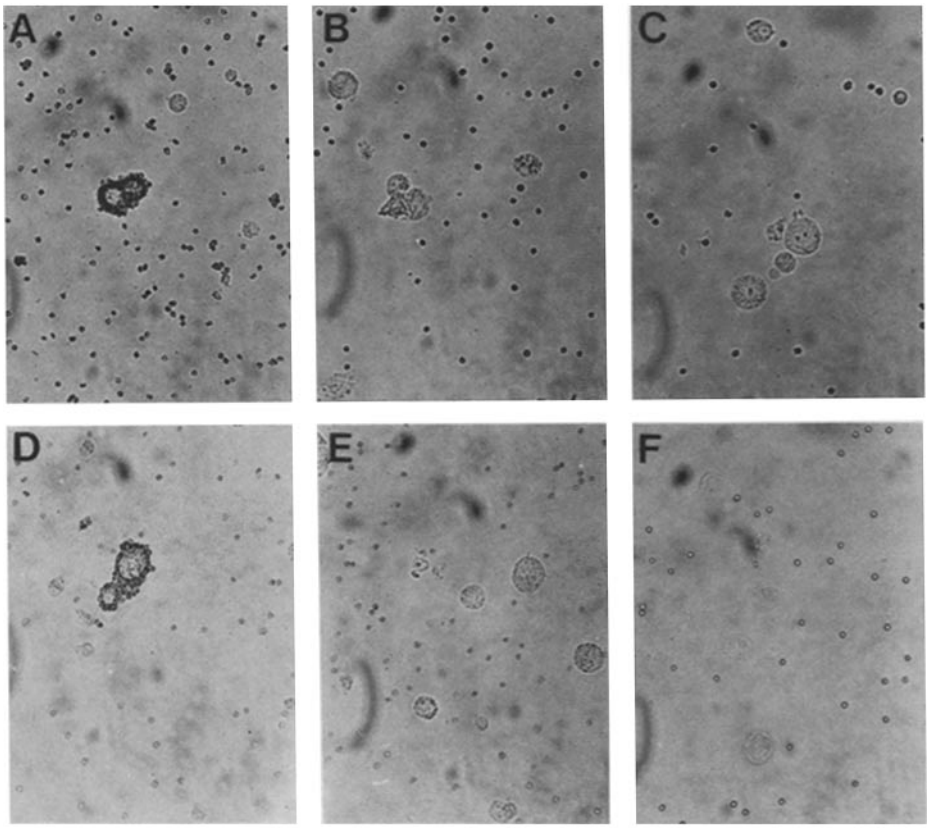
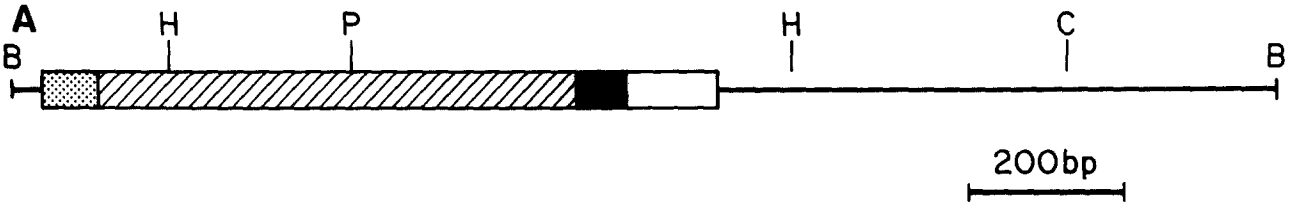


Figure 2. IgA-specific rosette formation by transfected cells. COS cells were transfected with pHuCFcαR plasmid (*A–E*) and incubated with human IgA-OE (*A–D*) or human IgG1-OE (*E*), and microscopically analyzed for rosette formation. In some cases, transfected cells were incubated with "blocking" antibodies before addition of OE: (*B*) My43; (*C*) HuIgA; (*D*) HuIgG. In *F*, COS cells transfected with control plasmid were incubated with IgA-OE.



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                GGCACAGATCTTGGAACGAGACGACCTGCTGTCAGCACG ATG GAC CCC AAA CAG ACC ACC CTC CTG TGT CTT GTG CTC TGT CTG GGC CAG   90
                                                        Met Asp Pro Lys Gln Thr Thr Leu Leu Cys Leu Val Leu Cys Leu Gly Gln  -5

AGG ATT CAG GCA CAG GAA GGG GAC TTT CCC ATG CCT TTC ATA TCT GCC AAA TCG AGT CCT GTG ATT CCC TTG GAT GGA TCT GTG AAA ATC  180
Arg Ile Gln Ala Gln Glu Gly Asp Phe Pro Met Pro Phe Ile Ser Ala Lys Ser Ser Pro Val Ile Pro Leu Asp Gly Ser Val Lys Ile   26

CAG TGC CAG GCC ATT CGT GAA GCT TAC CTG ACC CAG CTG ATG ATC ATA AAA AAC TCC ACG TAC CGA GAG ATA GGC AGA AGA CTG AAG TTT  270
Gln Cys Gln Ala Ile Arg Glu Ala Tyr Leu Thr Gln Leu Met Ile Ile Lys Asn Ser Thr Tyr Arg Glu Ile Gly Arg Arg Leu Lys Phe   56

TGG AAT GAG ACT GAT CCT GAG TTC GTC ATT GAC CAC ATG GAC GCA AAC AAG GCA GGG CGC TAT CAG TGC CAA TAT AGG ATA GGG CAC TAC  360
Trp Asn Glu Thr Asp Pro Glu Phe Val Ile Asp His Met Asp Ala Asn Lys Ala Gly Arg Tyr Gln Cys Gln Tyr Arg Ile Gly His Tyr   86

AGA TTC CGG TAC AGT GAC ACC CTG GAG CTG GTA GTG ACA GGC TTG TAT GGC AAA CCC TTC CTC TCT GCA GAT CGG GGT CTG GTG TTG ATG  450
Arg Phe Arg Tyr Ser Asp Thr Leu Glu Leu Val Val Thr Gly Leu Tyr Gly Lys Pro Phe Leu Ser Ala Asp Arg Gly Leu Val Leu Met  116

CCA GGA GAG AAT ATT TCC CTC ACG TGC AGC TCA GCA CAC ATC CCA TTT GAT AGA TTT TCA CTG GCC AAG GAG GGA GAA CTT TCT CTG CCA  540
Pro Gly Glu Asn Ile Ser Leu Thr Cys Ser Ser Ala His Ile Pro Phe Asp Arg Phe Ser Leu Ala Lys Glu Gly Glu Leu Ser Leu Pro  146

CAG CAC CAA AGT GGG GAA CAC CCG GCC AAC TTC TCT TTG GGT CCT GTG GAC CTC AAT GTC TCA GGG ATC TAC AGG TGC TAC GGT TGG TAC  630
Gln His Gln Ser Gly Glu His Pro Ala Asn Phe Ser Leu Gly Pro Val Asp Leu Asn Val Ser Gly Ile Tyr Arg Cys Tyr Gly Trp Tyr  176

AAC AGG AGC CCC TAC CTG TGG TCC TTC CCC AGT AAT GCC TTG GAG CTT GTG GTC ACA GAC TCC ATC CAC CAA GAT TAC ACG ACG CAG AAC  720
Asn Arg Ser Pro Tyr Leu Trp Ser Phe Pro Ser Asn Ala Leu Glu Leu Val Val Thr Asp Ser Ile His Gln Asp Tyr Thr Thr Gln Asn  206

TTG ATC CGC ATG GCC GTG GCA GGA CTG GTC CTC GTG GCT CTC TTG GCC ATA CTG GTT GAA AAT TGG CAC AGC CAT ACG GCA CTG AAC AAG  810
Leu Ile Arg Met Ala Val Ala Gly Leu Val Leu Val Ala Leu Leu Ala Ile Leu Val Glu Asn Trp His Ser His Thr Ala Leu Asn Lys  236

GAA GCC TCG GCA GAT GTG GCT GAA CCG AGC TGG AGC CAA CAG ATG TGT CAG CCA GGA TTG ACC TTT GCA CGA ACA CCA AGT GTC TGC AAG  900
Glu Ala Ser Ala Asp Val Ala Glu Pro Ser Trp Ser Gln Gln Met Cys Gln Pro Gly Leu Thr Phe Ala Arg Thr Pro Ser Val Cys Lys  266

TAAACACCTGGAGGTGAAGGCAGAGAGGAGCCAGGACTGTGGAGTCCGACAAAGCTACTTGAAGGACACAAGAGAGAAAAGCTCACTAAGAAGCTTGAATCTACTTTTTTTTTTTTTTG 1019

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TCTCACTCTGTTGCCCAGGAGGCGGAGGTTGCAGTGAACCAAGAGATGGCGCCAGTGCACTCCACCCTGGGTGACAGAGAGACTCTTTCTTTTTAAAAAAAAAAAAAAAAAAAAAA  1611
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Figure 3. Human FcαR cDNA. (*A*) Restriction map and schematic representation of FcαR. Restriction sites are indicated for the enzymes BglII (*B*), ClaI (*C*), HindIII (*H*), and PstI (*P*). The boxed coding region depicts the predicted domain structure, including signal sequence (*stippled*), extracellular (*crosshatched*), transmembrane (*black*), and intracellular (*white*) domains. (*B*) Human FcαR nucleotide and predicted amino acid sequences. Nucleotides are numbered beginning at 5′ terminus, and amino acids are numbered beginning with the predicted NH_2 terminus (Gln 22) of mature FcαR, which is marked with an arrowhead. Cysteine residues are boxed and potential N-linked glycosylation sites are marked with an asterisk. These sequence data are available from EMBL/Gen Bank/DDBJ under accession number X54150.

OE (Fig. 2 *A*), whereas control COS cells did not (Fig. 2 *F*). This activity was completely inhibited by the My43 mAb (Fig. 2 *B*) and human IgA (Fig. 2 *C*), but not by human IgG (Fig. 2 *D*). In a separate experiment, the inhibitory effects of My43 and human IgA were found to be dose dependent (Table 1). As a further indication of target specificity, the FcαR-transfected COS cells failed to form rosettes with human IgG-coated OE (Fig. 2 *E* and Table 1). Thus, the recombinant receptor is capable of initiating the first step in IgA-mediated immune effector functions, i.e., specific binding of IgA-coated targets.

The structural features of the 1.6-kb cDNA insert in pHuFcαR were determined by restriction endonuclease mapping (Fig. 3 *A*) and nucleotide sequence analysis (Fig. 3 *B*). The pHuFcαR cDNA is composed of a 39-bp 5′ untranslated region, a 861-bp open reading frame, and a 711-bp 3′ untranslated region terminating in a poly-A tract. The 3′ region also includes an Alu-sequence (1020–1296) (reference

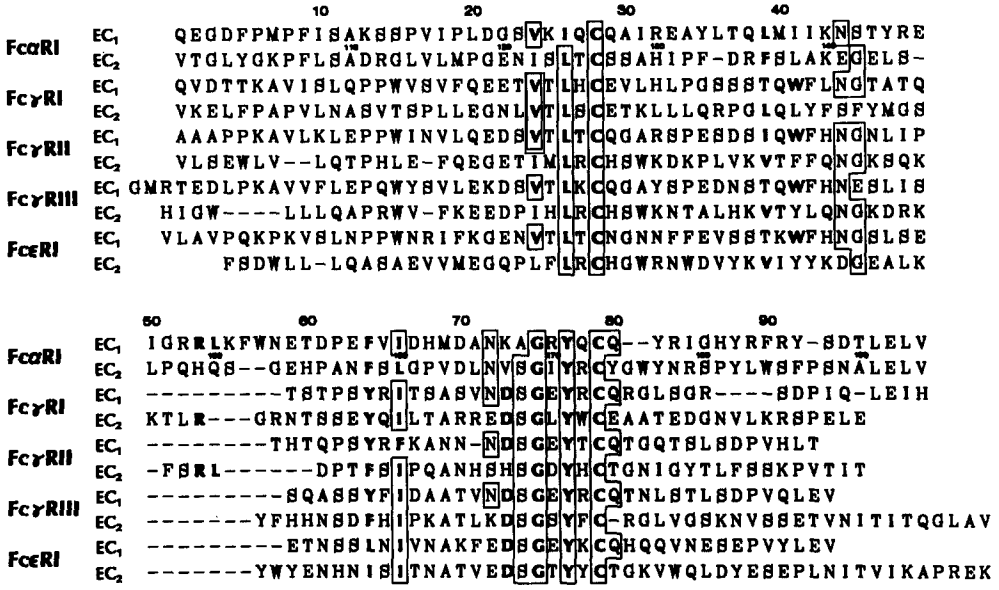

Figure 4. Alignment of FcαR Ig domains. Bold residues indicate conserved residues typical of Ig family members; boxed residues indicate FcαR domain residues common with five other FcR domains in this alignment; numbers indicate location of residues in the FcαR sequence. EC_1 and EC_2 denote first and second extracellular domains of the indicated FcR protein.

28) and AT-rich stretches, but lacks a polyadenylation signal. The open reading frame encodes a protein of 287 amino acids, the first 21 amino acids of which exhibit characteristics of a hydrophobic signal sequence (29). Thus, the predicted mature receptor sequence would begin at Gln22, resulting in a peptide of predicted M_r ~29,900, a value that is consistent with the reported molecular mass (32–36 kD; reference 13) of deglycosylated U937 FcαR. The 206-amino acid extracellular domain includes six potential sites for N-linked glycosylation, a feature that along with O-glycosylation could account for the significantly higher molecular mass, ~60 kD, observed for native FcαR.

When the amino acid sequence for the extracellular region was compared with existing protein sequence databases using the FASTA algorithm (30), homology was found with the other Ig FcRs sequenced to date. Further analysis of the FcR sequences using the NBRF program ALIGN (23) revealed statistically significant alignments between the FcαR and other members of this receptor family. Most notably, the FcαR appeared, as do the other FcRs, to contain several conserved residues that are the hallmarks of Ig-like sequences (31) in two contiguous domains. These residues, including the two cysteines in each domain that form the disulfide bond that holds together the Ig fold (31), are indicated in an align-

Table 2. *Alignment Scores and Percent Sequence Identity for the Ig Fc Receptor Family*

		FcαRI		FcγRI		FcγRII		FcγRIII		FcεRI	
Sequence		EC_1	EC_2	EC_1	EC_2	EC_1	EC_2	EC_1	EC_2	EC_1	EC_2
FcαRI	EC_1	–	7.0	3.4	4.1	4.5	5.5	4.6	3.6	2.4	3.1
	EC_2	23.2	–	4.8	5.2	5.1	4.2	4.0	3.5	3.6	2.6
FcγRI	EC_1	22.4	24.7	–	4.8	15.9	5.2	14.6	4.6	15.3	3.2
	EC_2	22.8	21.7	25.9	–	4.9	4.2	5.3	3.1	5.3	4.6
FcγRII	EC_1	24.1	25.3	49.4	26.5	–	6.0	17.2	5.6	13.0	3.4
	EC_2	20.0	28.2	28.2	18.8	24.1	–	4.7	17.1	3.9	13.7
FcγRIII	EC_1	28.2	21.2	49.4	28.2	55.4	21.2	–	4.5	12.3	3.1
	EC_2	18.2	20.5	27.1	18.2	25.3	45.9	22.4	–	4.3	13.1
FcεRI	EC_1	17.6	24.7	38.8	27.1	43.4	23.5	43.5	25.9	–	3.3
	EC_2	19.6	25.0	22.4	20.7	24.1	36.6	25.9	42.0	28.2	–

Scores are the SD for the indicated pair of aligned sequences (top) vs. the average scores from 100 randomized alignments of the same pair of sequences (bottom).

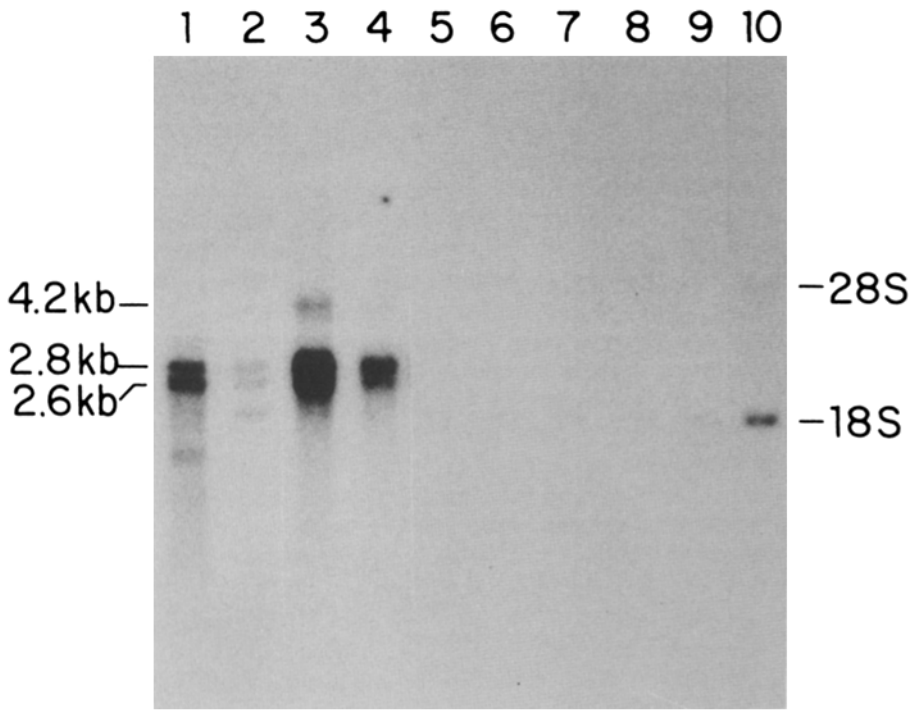

Figure 5. Autoradiogram of hybridization of FcαR to Northern blot of human myeloid and lymphoid cell mRNA. Polyadenylated RNA was analyzed from the following cells: (lane *1*) 12-h PMA-stimulated U937; (lane *2*) unstimulated U937; (lane *3*) neutrophils; (lane *4*) peripheral blood monocytes; (lane *5*) PWM-stimulated tonsillar B cells; (lane *6*) PMA/ionomycin-treated tonsillar B cells; (lane *7*) unstimulated tonsillar B cells; (lane *8*) PHA-stimulated tonsillar T cells; (lane *9*) unstimulated tonsillar T cells; and (lane *10*) total RNA from human dermal fibroblasts.

ment of the FcR sequences in Fig. 4. Given the conservation of Ig domain structure, it is likely that Cys 28 is disulfide bonded to Cys 79 in domain 1, and Cys 125 and Cys 172 are paired in domain 2 (shown as boxed residues in Fig. 4). The resulting ALIGN scores from the alignment are shown in Table 2. It is clear from these alignments that although the FcαR is homologous to the other known FcRs and is more closely related to those proteins than to several other members of the Ig superfamily, it is somewhat more distantly related to other FcRs than are these receptors to each other. Thus, it is likely that the FcαR diverged from a common ancestor very early in the development of the Ig FcR gene family. The existence of a distinct family of FcRs is supported by previous work demonstrating that the genes for FcγRI, FcγRII, FcγRIII, and the α chain of FcεRI map to the long arm of chromosome 1 (32, 33). We are currently investigating the possibility that the FcαR, which shares structural and functional qualities with these other FcRs, maps to the same chromosomal region.

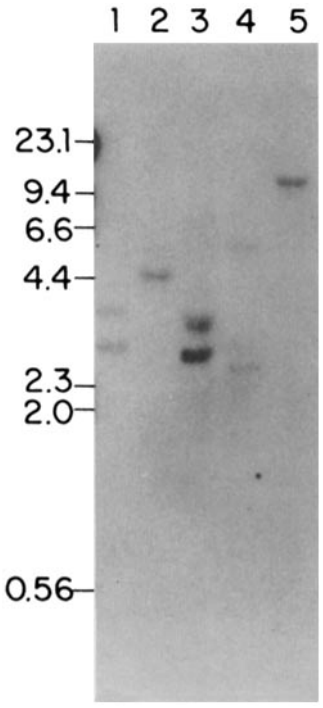

Figure 6. Autoradiogram of hybridization of FcαR cDNA probe to Southern blot of human genomic DNA. Genomic DNA was digested with EcoRI (lane *1*), HindIII (lane *2*), BamHI (lane *3*), PstI (lane *4*), or BglII (lane *5*).

Hydropathicity analysis indicated that the extracellular region of the FcαR is followed by a 19-residue stretch of hydrophobic amino acids (Leu 207 to Val 225) corresponding to a potential transmembrane region. The intracytoplasmic region would thus be composed of 41 amino acids. Two features of the putative transmembrane region are atypical of "protein anchored" transmembrane proteins, including the presence of a charged residue (Arg 230) within the hydrophobic transmembrane domain and the lack of a cluster of basic residues immediately following the hydrophobic stretch (34). A number of proteins containing a charged residue in the transmembrane region have been reported to attach to the membrane either through a glycosyl-phosphatidyl inositol (GPI) linkage (e.g., the Qa-2 MHC antigen; reference 35) or by association with another membrane-bound protein (e.g., the TCR, which requires association with the CD3 complex for cell surface expression; reference 36). With regard to Fc receptors, the α chain of the high affinity IgE receptor (FcεRI) contains an Asp residue in the transmem-

brane region and is attached by the latter mechanism, i.e., through interaction with the γ chain (37). The two genes for human FcγRIII also include an Asp residue in the middle of the membrane-spanning region (38). The FcγRIII isoform expressed by PMNs is GPI linked (38, 39), while the isoform expressed by NK cells and macrophages appears to require an additional peptide for cell surface expression, a function that can be provided by the γ chain of the FcϵRI (40, 41). The critical difference between these two isoforms appears to reside in the presence or absence of a Ser 203 residue in the extracellular region, which signals GPI attachment (40–42). Although there is a Ser residue in an analogous site of the FcαR (Ser 197), it is unlikely that FcαR is GPI linked, since Monteiro et al. (13) have shown that FcαR on human monocytes, granulocytes, and U937 cells are resistant to cleavage with GPI-specific phospholipase C (PLC). We have made similar observations with these cell populations, and also found that the level of expression of FcαR on transfected COS cells was unaffected by PLC treatment. The presence of an associated protein cannot be ruled out, although results with transfected COS cells would require such a protein to be present in both myeloid cells and COS-7 cells. Clearly, additional studies are required to determine the nature of FcαR attachment and the possible relevance of the different forms of membrane attachment of Fc receptor function.

Using an RNA probe representing the 5′ untranslated region and coding sequence for a portion of the extracellular region, we analyzed expression of FcαR mRNA (Fig. 5). Two major hybridizing transcripts of ~2.8 and ~2.6 kb were observed in polyadenylated RNA preparations from U937 cells, peripheral blood monocytes, and PMN. PMA-stimulated U937 cells expressed significantly higher FcαR message levels in comparison with unstimulated U937 cells, which is consistent with the finding that U937 cell surface FcαR protein expression is PMA inducible (13). Subpopulations of human B and T lymphocytes have been reported to express cell surface FcαR (43–45). Except for nonspecific binding to ribosomal RNA, the FcαR probe failed to hybridize with polyadenylated RNA from either untreated or mitogen-stimulated tonsillar B or T cells (Fig. 5, lanes *5–9*), even after extended exposure of blots. This finding would suggest either that the receptor on these cells is structurally distinct or that the conditions used failed to induce receptor expression.

Human genomic DNA was digested with five different restriction endonucleases and subjected to Southern blot analysis, using as probe a ^{32}P-labeled cDNA fragment representing the 5′ untranslated and extracellular regions of the FcαR cDNA (Fig. 6). Single or double hybridizing bands were observed for each restriction digest, suggesting that the human myeloid FcαR gene exists as a single copy.

In conclusion, we have isolated a cDNA clone encoding a cell surface protein that displays IgA binding properties and that is structurally similar to FcRs for other isotypes. The availability of this cDNA will facilitate investigations into such issues as genomic organization, regulation of gene expression, and structure/function relationships. Furthermore, a plasmid that directs the synthesis of recombinant soluble FcαR was recently constructed (Maliszewski, C., unpublished results). Clearly, such a reagent will be valuable in dissecting the role of FcαR in IgA-mediated effector functions and in immunoregulation of IgA synthesis.

We thank Alan Alpert for FACS analysis; Tim Sato, Cristy McNutt, and Michael Comeau for expert technical assistance; Drs. Steven Gillis, David Cosman, Steven Dower, and Kenneth Grabstein for critical review of the manuscript; and Dr. Michael Fanger for helpful discussions.

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Received for publication 30 July 1990.

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