

Expression Cloning of a Human Fc Receptor for IgA

By Charles R. Maliszewski,* Carl J. March,*
Michael A. Schoenborn,* Steve Gimpel,* and Li Shen†

From the *Immunex Corporation, Seattle, Washington 98101; and the †Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03756

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 (Fc α R) have been implicated in a variety of immune effector mechanisms, the molecular features of Fc α R 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 Fc α R sequence is unique, is a member of the immunoglobulin gene superfamily, and is related to Fc receptors for IgG (Fc γ RI, II, and III) and IgE (Fc ϵ RI).

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 (Fc α R) on effector cells. In particular, human monocytes, macrophages, neutrophils, and myeloid cell lines express cell surface Fc α R (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 Fc α R is a protein of \sim 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 isolymp (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 μ g/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 μ g/ml; Sigma Chemical Co.), PWM (40 μ g/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 BglIII 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). Fc α R⁺ cells were selected by incubation in the My43 anti-Fc α R 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-Fc α R (My43) binding to transfected cells. Individual clones containing Fc α R cDNA were isolated from positive pools. One of these, pHuFc α R, 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-Fc α R 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-Fc α R 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 μ g/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 μ l) were mixed for 16 h at 10°C with 25 μ l 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 \times Denhardt's solution, 5 \times SSC, 0.1% SDS, 50 mM KH₂PO₄, 20 mM EDTA, 0.05% *N*-lauroylsarcosine, 150 μ g sheared, denatured salmon sperm DNA per ml) containing ³²P-labeled antisense riboprobe transcribed from the 5' portion (\sim 430 bases) of pHuFc α R. Washes were carried out in 2 \times 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 μ g 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 ³²P-labeled 700-bp fragment encoding the 5' untranslated and extracellular coding region of Fc α R. Hybridized blots were washed in 0.2 \times 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 Fc α R 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 Fc α R expression as measured by My43 binding is inducible by PMA treatment. We found that maximal Fc α R 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, Fc α R-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 Fc α R 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 pHuFc α R, 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

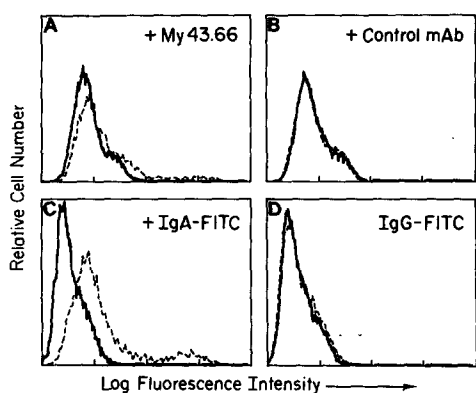


Figure 1. Expression of Fc α R cDNA in transfected cells. (A and B) Anti-Fc α R binding. Control plasmid (solid line) and pHuFc α R (dashed line) transfected COS cells were stained with either My43 or a murine IgM isotype control and analyzed by flow cytometry. (C and D) IgA binding. Control plasmid and pHuFc α R 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, Fc α R-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 Fc α R⁺-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 μ g/ml	0.9	
	222 μ g/ml	6.2	(0.3)
	74 μ g/ml	9.3	(1)
Human IgG	2 mg/ml	9.7	(0.05)

Fc α R⁺ 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.

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

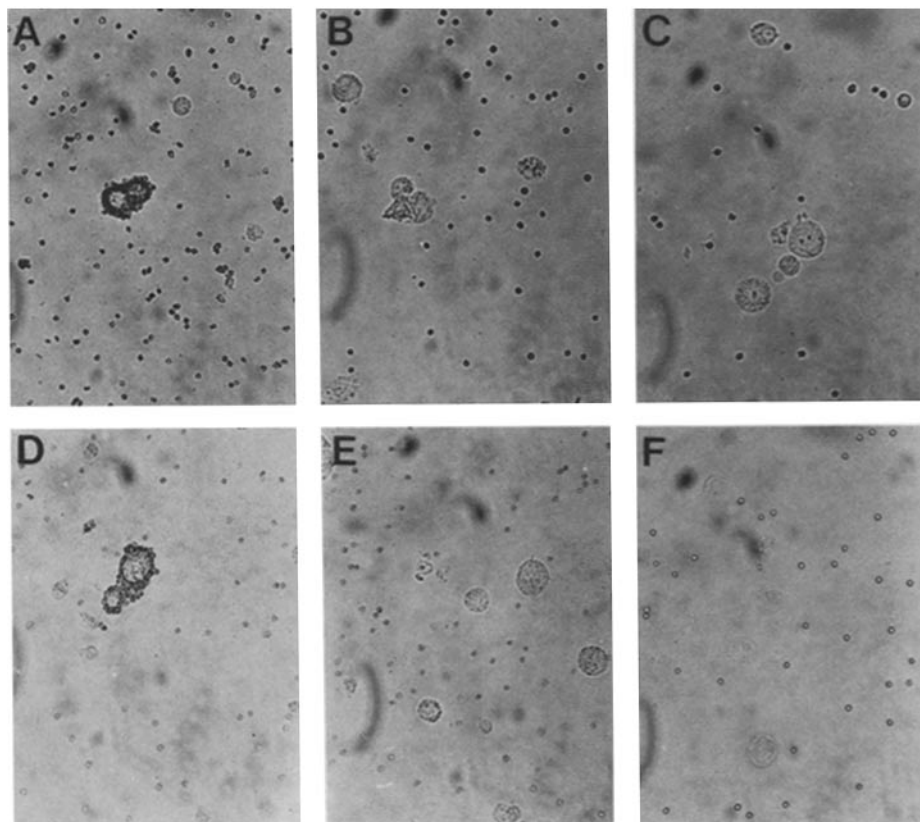
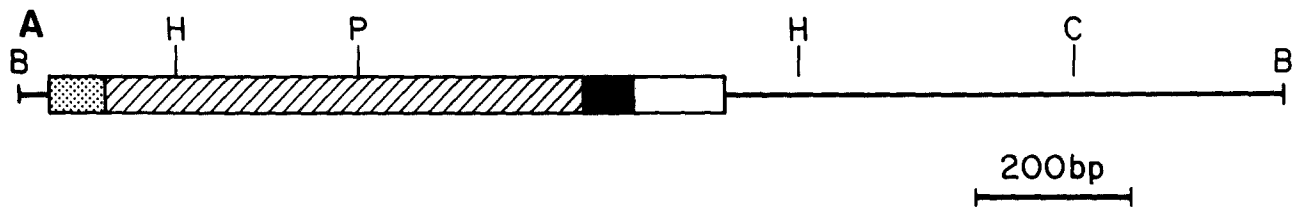


Figure 2. IgA-specific rosette formation by transfected cells. COS cells were transfected with pHuCFc α R plasmid (A-E) and incubated with human IgA-OE (A-D) or human IgG1-OE (E), and microscopically analyzed for rosette formation. In some cases, transfected cells were incubated with "blocking" antibodies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



B

```

GGCACAGATCTTGGAACGAGACGACCTGCTGTCTCAGCACG  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 Ser 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 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
TAAACACCTGGAGGTGAAGGCAGAGAGGAGCCAGGACTGTGGAGTCCGACAAAGCTACTTGAAGGACACAGAGAGAAAAGCTCACTAAGAAGCTTGAATCTACTTTTTTTTTTTTTTTT  1019
AGACAGAGTCTGGCTCTGTCCACCCAGGCTGAAGTGCAGTGGAGCAATCTCGGCTCATTGAACTCTTGGGTTCAAGTGATTCTGTGCCTCAGCCTCCCAAGTAGCTGGAATTACAGGC  1138
ACATACCCTGACCCAGCTAATTTTTGTATTTTTAGTAGAGATGGGTTTCACTGTGTGGCCAGGCTGGTCTCGAACTCTGGACCTCAGGTGATCCACCCACCTTGGCCCTCCCAA  1257
GTGCTGAGATTATAGGCATGAGCCACCACGCTGGCCAGATGCATGTTCAAACCAATCAAATGGTGTGTTTCTTATGCAGGACTGATCGATTTGCACCCACCTTCTGCACATAAGTTAT  1376
GGTTTTCCATCTTATCTGTCTTCTGATTTTTATATCCCTGTTAATTTCTCTCCTCATGTTCTCTCTTTTTTATTATTTTATTATTTTTATTTTTATTGAGACAGAG  1495
TCTCACTCTGTTGCCAGGAGGGGAGGTTGCAAGAGATGGCGCCAGTGCCTCCACCTGGGTGACAGAGAGACTCTTCTTTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1611

```

Figure 3. Human Fc α R cDNA. (A) Restriction map and schematic representation of Fc α R. 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 Fc α R nucleotide and predicted amino acid sequences. Nucleotides are numbered beginning at 5' terminus, and amino acids are numbered beginning with the predicted NH₂ terminus (Gln 22) of mature Fc α R, 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 Fc α R-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 pHuFc α R were determined by restriction endonuclease mapping (Fig. 3 A) and nucleotide sequence analysis (Fig. 3 B). The pHuFc α R 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

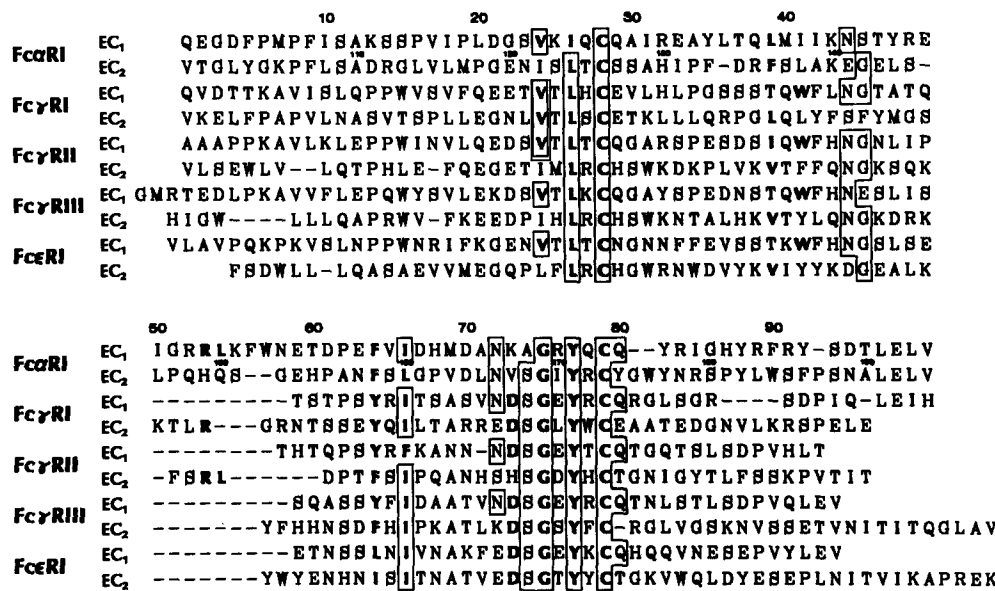


Figure 4. Alignment of Fc α R Ig domains. Bold residues indicate conserved residues typical of Ig family members; boxed residues indicate Fc α R domain residues common with five other FcR domains in this alignment; numbers indicate location of residues in the Fc α R sequence. EC₁ and EC₂ denote first 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 $M_r \sim 29,900$, a value that is consistent with the reported molecular mass (32–36 kD; reference 13) of deglycosylated U937 Fc α R. 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 Fc α R.

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 Fc α R and other members of this receptor family. Most notably, the Fc α R 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

Sequence	Fc α RI		Fc γ RI		Fc γ RII		Fc γ RIII		Fc ϵ RI		
	EC ₁	EC ₂	EC ₁	EC ₂	EC ₁	EC ₂	EC ₁	EC ₂	EC ₁	EC ₂	
Fc α RI	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
Fc γ RI	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
Fc γ RII	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
Fc γ RIII	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
Fc ϵ RI	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).

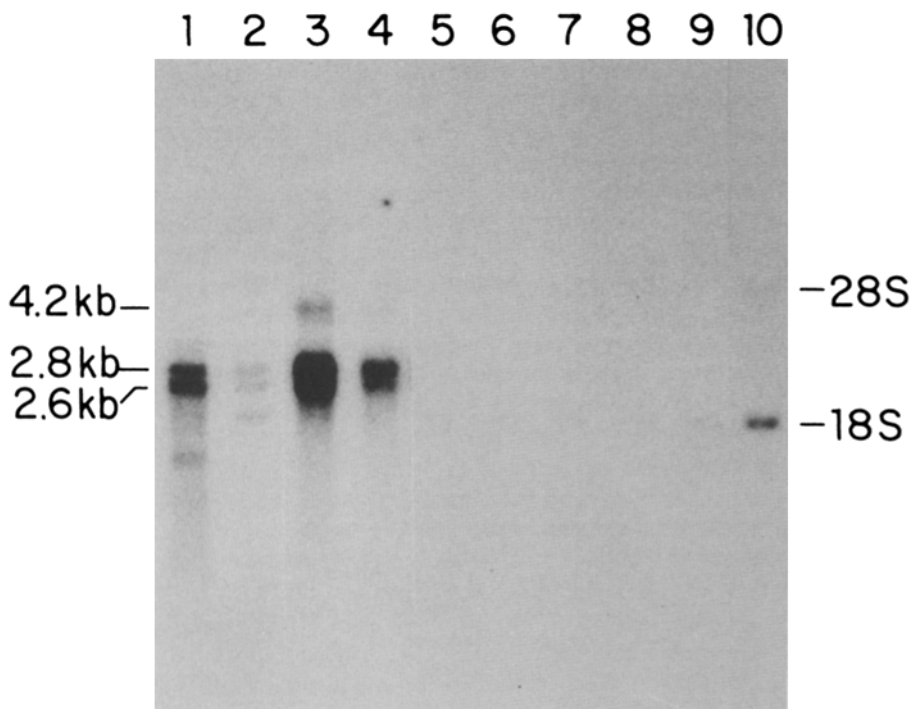


Figure 5. Autoradiogram of hybridization of Fc α R to Northern blot of human myeloid and lymphoid cell mRNA. Polyadenylated RNA was analyzed from the following cells: (lane 1) 12-h PMA-stimulated U937; (lane 2) unstimulated U937; (lane 3) neutrophils; (lane 4) peripheral blood monocytes; (lane 5) PWM-stimulated 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 Fc α R 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 Fc α R 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 Fc γ RI, Fc γ RII, Fc γ RIII, and the α chain of Fc ϵ RI map to the long arm of chromosome 1 (32, 33). We are currently investigating the possibility that the Fc α R, which shares structural and functional qualities with these other FcRs, maps to the same chromosomal region.

Hydropathicity analysis indicated that the extracellular region of the Fc α R 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 (Fc ϵ RI) contains an Asp residue in the transmem-

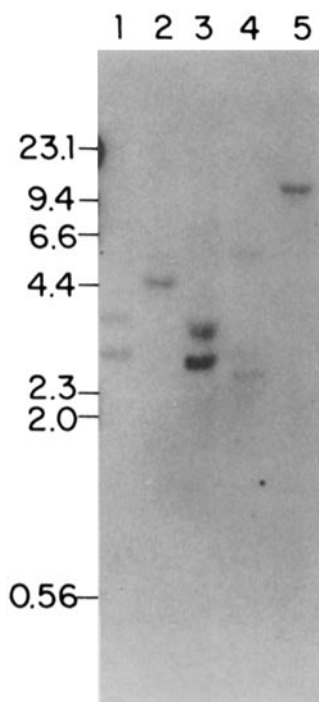


Figure 6. Autoradiogram of hybridization of Fc α R cDNA probe to Southern blot of human genomic DNA. Genomic DNA was digested with EcoRI (lane 1), HindIII (lane 2), BamHI (lane 3), PstI (lane 4), or BglII (lane 5).

brane region and is attached by the latter mechanism, i.e., through interaction with the γ chain (37). The two genes for human Fc γ RIII also include an Asp residue in the middle of the membrane-spanning region (38). The Fc γ RIII 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 Fc ϵ RI (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 Fc α R (Ser 197), it is unlikely that Fc α R is GPI linked, since Monteiro et al. (13) have shown that Fc α R 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 Fc α R 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 Fc α R 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 Fc α R mRNA (Fig. 5). Two major hybridizing transcripts of \sim 2.8 and \sim 2.6 kb were observed in polyadenylated RNA preparations from U937 cells, peripheral blood monocytes, and PMN. PMA-stimulated

U937 cells expressed significantly higher Fc α R message levels in comparison with unstimulated U937 cells, which is consistent with the finding that U937 cell surface Fc α R protein expression is PMA inducible (13). Subpopulations of human B and T lymphocytes have been reported to express cell surface Fc α R (43–45). Except for nonspecific binding to ribosomal RNA, the Fc α R 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 Fc α R cDNA (Fig. 6). Single or double hybridizing bands were observed for each restriction digest, suggesting that the human myeloid Fc α R 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 Fc α R was recently constructed (Maliszewski, C., unpublished results). Clearly, such a reagent will be valuable in dissecting the role of Fc α R 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.

Address correspondence to Charles R. Maliszewski, Department of Immunology, Immunex Corporation, 51 University Street, Seattle, WA 98101.

Received for publication 30 July 1990.

References

1. Underdown, B.J., and J.M. Schiff. 1986. Immunoglobulin A: strategic defense initiative at the mucosal surface. *Annu. Rev. Immunol.* 4:389.
2. Fanger, M.W., L. Shen, J. Pugh, and G.M. Bernier. 1980. Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. *Proc. Natl. Acad. Sci. USA.* 77:3640.
3. Gauldie, J., C. Richards, and L. Lamontagne. 1983. Fc receptors for IgA and other immunoglobulins on resident and activated alveolar macrophages. *Mol. Immunol.* 20:1029.
4. Maliszewski, C.R., L. Shen, and M.W. Fanger. 1985. The expression of receptors for IgA on human monocytes and calcitriol-treated HL-60 cells. *J. Immunol.* 135:3878.
5. Chevalier, A., R.C. Monteiro, H. Kubagawa, and M.D. Cooper. 1989. Immunofluorescence analysis of IgA binding by human mononuclear cells in blood and lymphoid tissue. *J. Immunol.* 142:2244.
6. Fanger, M.W., S.N. Goldstine, and L. Shen. 1983. Cytofluorographic analysis of receptors for IgA in human polymorphonuclear cells and monocytes and the correlation of receptor expression with phagocytosis. *Mol. Immunol.* 20:1019.
7. Shen, L., C.R. Maliszewski, W.F.C. Rigby, and M.W. Fanger. 1986. IgA-mediated effector function of HL-60 cells following treatment with calcitriol. *Mol. Immunol.* 23:611.
8. Yeaman, G.R., and M.A. Kerr. 1987. Opsonization of yeast

- by human serum IgA anti-mannan antibodies and phagocytosis by human polymorphonuclear leucocytes. *Clin. Exp. Immunol.* 68:200.
9. Shen, L., and M.W. Fanger. 1981. Secretory IgA antibodies synergize with IgG in promoting ADCC by human polymorphonuclear cells, monocytes, and lymphocytes. *Cell Immunol.* 59:75.
 10. Ferreri, N.R., W.C. Howland, and H.L. Spiegelberg. 1986. Release of leukotrienes C₄ and B₄ and prostaglandin E₂ from human monocytes stimulated with aggregated IgG, IgA, and IgE. *J. Immunol.* 136:4188.
 11. Gorter, A., P.S. Hiemstra, P.C.J. Leijh, M.E. van der Sluys, M.T. van der Barselaar, L.A. van Es, and M.R. Daha. 1987. IgA and secretory IgA-opsonized *S. aureus* induce a respiratory burst and phagocytosis by polymorphonuclear leukocytes. *Immunology.* 61:303.
 12. Albrechtson, M., G.R. Yeaman, and M.A. Kerr. 1988. Characterization of the IgA receptor from human polymorphonuclear leucocytes. *Immunology.* 64:201.
 13. Monteiro, R.C., H. Kubagawa, and M.D. Cooper. 1990. Cellular distribution, regulation, and biochemical nature of an Fc α receptor in humans. *J. Exp. Med.* 171:597.
 14. Shen, L., R. Lasser, and M.W. Fanger. 1989. My43, a monoclonal antibody that reacts with human myeloid cells inhibits monocyte IgA binding and triggers function. *J. Immunol.* 143:4117.
 15. Sundstrom, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int. J. Cancer.* 17:565.
 16. Saxon, A., J. Feldhaus, and R.A. Dobbins. 1976. Single step separations of human T and B cells using AET-treated SRBC rosettes. *J. Immunol. Methods.* 12:285.
 17. Gubler, V., and B. Hoffman. 1983. A simple and efficient method for generating cDNA libraries. *Gene (Amst.)* 25:263.
 18. Mosley, B., M.P. Beckmann, C.J. March, R.L. Idzerda, S.D. Gimpel, T. VandenBos, D. Friend, A. Alpert, D. Anderson, J. Jackson, J.M. Wignall, C. Smith, B. Gallis, J.E. Sims, D. Urdal, M.B. Widmer, D. Cosman, and L.S. Park. 1989. The murine interleukin-4 receptor: molecular cloning and characterization of secreted and membrane bound forms. *Cell.* 59:335.
 19. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
 20. Cosman, D., D.P. Cerretti, A. Larsen, L. Park, C. March, S. Dower, S. Gillis, and D. Urdal. 1984. Cloning, sequencing, and expression of human interleukin-2 receptor. *Nature (Lond.)* 312:768.
 21. Seed, B., and A. Aruffo. 1987. Molecular cloning of the CD2 antigen, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. *Proc. Natl. Acad. Sci. USA.* 84:3365.
 22. Hattori, M., and Y. Sakaki. 1986. Dideoxy sequencing method using denatured plasmid templates. *Anal. Biochem.* 152:232.
 23. Dayhoff, M.O., W.C. Barker, and L.T. Hunt. 1983. Establishing homologies in protein sequences. *Methods Enzymol.* 91:524.
 24. Karpovsky, B., J.A. Titus, D.A. Stephany, and D.M. Segal. 1984. Production of target-specific effector cells using hetero-cross-linked aggregates containing anti-target cell and anti-Fc receptor antibodies. *J. Exp. Med.* 160:1686.
 25. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156.
 26. Kroczeck, R.A., and E. Siebert. 1990. Optimization of Northern analysis by vacuum-blotting, RNA-transfer visualization, and ultraviolet fixation. *Anal. Biochem.* 184:90.
 27. Blin, N., and D.W. Stafford. 1976. Isolation of high molecular weight DNA. *Nucleic Acids. Res.* 3:2303.
 28. Schmid, C.W., and W.R. Jelinek. 1982. The Alu family of dispersed repetitive sequences. *Science (Wash. DC).* 216:1065.
 29. von Heijne, G. 1986. A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* 14:4683.
 30. Devereux, J., P. Haeblerli, and O. Smithies. 1984. A complex set of sequence analysis programs for the VAX. *Nucleic Acid Res.* 12:387.
 31. Williams, A.F., and A.N. Barclay. 1988. The immunoglobulin superfamily-domains for cell surface recognition. *Annu. Rev. Immunol.* 6:381.
 32. Huppi, K., B.A. Mock, J. Hilgers, J. Kochan, and J.-P. Kinet. 1988. Receptors for Fc ϵ and Fc γ are linked on mouse chromosome I. *J. Immunol.* 141:2807.
 33. Grundy, H.O. 1989. The polymorphic Fc gamma receptor II gene maps to human chromosome 1q. *Immunogenetics.* 29:331.
 34. Ferguson, M.A.J., and A.F. Williams. 1988. Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. *Annu. Rev. Biochem.* 57:285.
 35. Clevers, H., B. Alarcon, T. Wileman, and C. Terhorst. 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol.* 6:629.
 36. Waneck, G.L., E. Stein, and R.A. Flavell. 1988. Conversion of PI-anchored protein to an integral membrane protein by a single amino acid mutation. *Science (Wash. DC).* 241:697.
 37. Blank, U., C. Ra, L. Miller, K. White, H. Metzger, and J.P. Kinet. 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature (Lond.)* 337:187.
 38. Ravetch, J.V., and B. Perussia. 1989. Alternative membrane forms for Fc γ RIII(CD16) of human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. *J. Exp. Med.* 170:481.
 39. Simmons, D., and B. Seed. 1988. The Fc γ receptor of natural killer cells is a phospholipid-linked membrane protein. *Nature (Lond.)* 339:568.
 40. Kurosaki, T., and J.V. Ravetch. 1989. A single amino acid in the glycosyl phosphatidylinositol attachment domain determines the membrane topology of Fc γ RIII. *Nature (Lond.)* 342:805.
 41. Hibbs, M.L., P. Selvaraj, O. Carpen, T.A. Springer, H. Kuster, M.-H.E. Jouvin, and J.-P. Kinet. 1989. Mechanisms for regulating expression of membrane isoforms of Fc γ RIII (CD16). *Science (Wash. DC).* 246:1608.
 42. Lanier, L.L., S. Cwirla, G. Yu, R. Testi, and J.H. Phillips. 1989. Membrane anchoring of a human IgG Fc receptor (CD16) determined by a single amino acid. *Science (Wash. DC).* 246:1611.
 43. Gupta, S., C.D. Platsoucas, and R.A. Good. 1979. Receptors for IgA on a subpopulation of human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 76:4025.
 44. Lum, L.G., A.V. Muchmore, D. Keren, J. Decker, I. Koski, W. Strober, and R.M. Blaese. 1979. A receptor for IgA on human T lymphocytes. *J. Immunol.* 122:65.
 45. Fanger, M.W., and P.M. Lydyard. 1981. Receptors for IgA on human lymphocytes. I. Detection and specificity. *Mol. Immunol.* 18:189.