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Isolation of cDNAs for two distinct human Fc receptors by ligand affinity cloning

Siegfried Stengelin, Ivan Stamenkovic and Brian Seed

Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA

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Two cDNA clones encoding different but related receptors for immunoglobulin G constant domains were isolated from cDNA expression libraries by a ligand-mediated selection procedure ('affinity cloning'). Because both of the receptors encoded by the cDNAs react with CDw32 monoclonal antibodies, and both show the appropriate IgG binding affinity, both appear to be forms of the receptor formerly thought to be a single species called FcRII. The extracellular domains encoded by the isolated clones are closely related to the murine IgG2b/1 β receptor extracellular domains, but the intracellular domains are unrelated. The receptors expressed in COS cells show a preference for IgG1 among IgG subtypes and no affinity for IgM, IgA or IgE. Abundant expression of the RNAs was detected in myeloid cell lines and placenta.

Key words: FcRII/CDw32/expression library/IgG receptor subtypes

Introduction

Three human leukocyte receptors for immunoglobulin G constant domains, FcRI, FcRII and FcR_{lo}, have been identified by biochemical studies (reviewed by Burton, 1985; Anderson and Looney, 1986). The latter two have been given cluster of differentiation assignments CDw32 and CD16 respectively (McMichael *et al.*, 1987). CDw32 is found on macrophages, granulocytes, platelets and B lymphocytes, while CD16 is found on macrophages, neutrophils, large granular lymphocytes and a subset of T cells thought to comprise the suppressor cells. Both CD16 and CDw32 receptors have low affinity for IgG ($K_a \sim 10^6$ M^{-1}), while FcRI has a much higher affinity ($K_a \sim 10^8$ M^{-1}). It has been suggested that human FcRI and FcRII correspond to the murine IgG2a and IgG2b/1 receptors respectively (Anderson and Looney, 1986). To date three distinct murine FcγR cDNAs have been isolated, FcγRs α, β1 and β2, all apparently encoding IgG2b/1 receptors (Ravetch *et al.*, 1986; Lewis *et al.*, 1986; Hibbs *et al.*, 1986). In this report two closely related CDw32 receptor cDNAs were isolated from COS cell expression libraries by an affinity selection technique.

Results

Isolation of the cDNAs

Two distinct Fc receptor clones were isolated from cDNA libraries prepared from human pheochromocytoma and thyroid carcinoma biopsy samples. The libraries were introduced into COS cells by spheroplast fusion, allowed to replicate and express the cDNA inserts for 48 h, detached, treated with mouse or human IgG antibodies, and allowed to settle onto dishes coated with affinity purified sheep anti-mouse IgG or goat anti-human IgG antibodies. After lysis, DNA recovery and transformation into *Escherichia coli*, the cycle was repeated for two more rounds, whereupon individual colonies were selected, plasmid DNA was prepared from them by the alkaline miniprep method (Maniatis *et al.*, 1982), and the resulting DNA was transformed into COS cells using DEAE dextran as a facilitator (Seed and Aruffo, 1987). About 50% of the transfected cultures gave a weak positive reaction by indirect immunofluorescence. A 2.3 kb insert was observed in all positive clones obtained from the pheochromocytoma library and a 2.2 kb insert was similarly observed among positive clones from the thyroid carcinoma library. A single clone was selected from each library for further study, PC23 from the pheochromocytoma library, and TC9 from the thyroid carcinoma library.

Antibody binding specificity

Indirect immunofluorescence studies showed that the two receptors expressed on COS cells bound all mouse and human IgGs with low affinity and a discrimination among human antibodies for IgG1. Human IgM, IgA1, IgA2 and IgE did not bind, nor did murine IgM or IgA. As expected, human Fc, but not Fab fragments bound to the transfected cells, but the affinity of the Fc fragments appeared substantially lower than the affinity of intact IgG. Among monoclonal antibodies donated to the Third International Workshop on Leukocyte Differentiation Antigens, three gave strong positive immunofluorescence: two (CIKM5 and 2E1) recognizing the Fc receptor CDw32 determinant, and one out of four (PL-13) recognizing a CD23 (B cell IgE Fc receptor) determinant (tested only on the PC23 clone). Monoclonals recognizing the T cell/macrophage Fc receptor antigen CD16 gave only weak immunofluorescence comparable with that shown by monoclonal antibodies recognizing other antigens. Antibody KB61, recently suggested to recognize a B-cell form of the CD32 molecule (Tetteroo *et al.*, 1987), did not react with either TC9 or PC23 transfected cells (D.Simmons, personal communication).

Scatchard analysis of the binding of radiolabelled IgG1 to transfected COS cells showed the presence of $2-4 \times 10^6$ sites per cell with an association constant of $\sim 2 \times 10^6$ l/mol for the PC23 receptor and $\sim 10^6$ l/mol for the TC9 receptor (Figure 1). A ligand displacement titration with IgG2, 3 and 4 (Figure 2) gave association constants for IgG2 and IgG4 of $\sim 5 \times 10^5$ l/mol with PC23 and $\sim 3 \times 10^5$ l/mol with TC9 calculated by the method of Cheng and Prusoff (1973). The IgG3 titration could not be completed due to low binding affinity, but an association constant of $< 10^5$ l/mol could be inferred.

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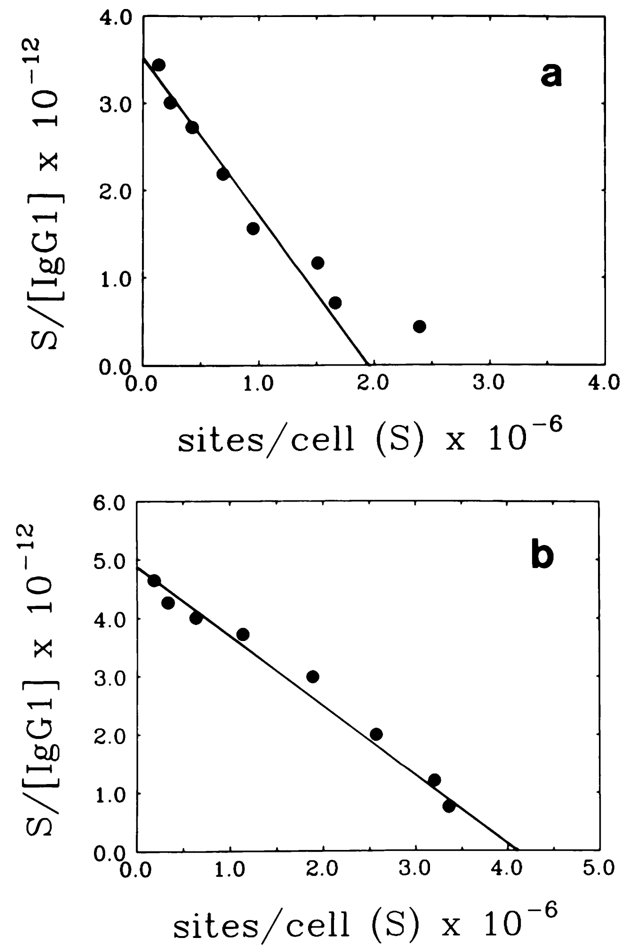

Fig. 1. Scatchard analysis of IgG1 binding to COS cells transfected with the PC23 (a) or TC9 (b) cDNAs. Binding of radiolabelled IgG1 to transfected cells was carried out at 4°C as described in Materials and methods. Data were corrected for the contribution of nonspecific (nonsaturable) binding by subtraction of the c.p.m. bound in the presence of 2.5×10^{-4} M total IgG.

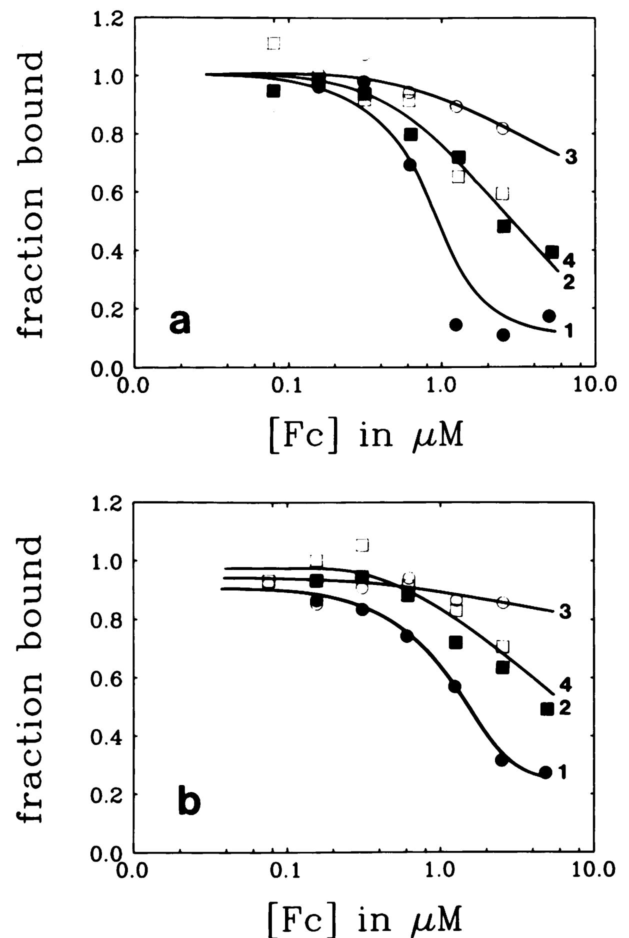

Fig. 2. Ligand displacement analysis of the binding of IgG subtypes to PC23 (a) and TC9 (b) receptors. The fraction of ^{125}I-labelled IgG1 tracer bound in the presence of nonradioactive IgG1, 2, 3 and 4 is plotted as a function of the Fc concentration of the competitor. ●, IgG1; ■, IgG2; ○, IgG3; □, IgG4.

IgG adsorption and immunoprecipitations

Lysates of surface labelled cells were allowed to adsorb to human IgG immobilized on agarose and then washed as for immunoprecipitation. Electrophoresis of material adsorbed from lysates of COS cells transfected with the PC23 clone showed a broad band of mol. wt ~35 kd (Figure 3) which resolved into two bands of 34 and 36 kd on short exposure (not shown). The material adsorbed from lysates of TC9 transfected cells was smaller, ~33 kd. HL-60 cells induced with phorbol ester gave rise to a broad band of ~39 kd, resolving into two bands of 38 and 40 kd on short exposure (not shown). The smaller size of the cDNA products expressed by transfected cells presumably reflects a reduced level of glycosylation; a similar diminution of mass in COS cells has been observed with other surface antigens (Seed and Aruffo, 1987; Aruffo and Seed, 1987a,b; Seed, 1987). Two faint bands possibly corresponding to the TC9 form of the Fc receptor were also found in the material adsorbed from lysates of HL-60 cells (Figure 3).

Radioimmunoprecipitation of transfected cells and phorbol ester-induced HL-60 cells with a mixture of CDw32 antibodies gave similar results, albeit with lower resolution (Figure 3). An ~40 kd protein was observed in lysates prepared from phorbol ester-induced U937 cells, although in low abundance (not shown).

RNA blot analysis

RNA blot analysis with a PC23 probe showed three major RNAs of 2.4, 1.6 and 1.0 kb (Figure 4). The largest transcript was prominent in RNA from the macrophage-like lines HL-60 and U937, as well as placenta. Rehybridization of the filter showed that another macrophage specific surface antigen transcript was also highly expressed in placenta (not shown), supporting the notion that placental macrophages (Wood *et al.*, 1982; Uren and Boyle, 1985) were primarily responsible for the strong signal observed. Rehybridization of the filter with a TC9 probe gave essentially the same pattern as observed with the PC23 probe, demonstrating that the smallest transcript contained at least a portion of the coding sequence.

Nucleotide sequence of the cDNAs

The nucleotide sequence of the PC23 clone (Figure 5) is long enough to represent a full-length or near full-length copy of the largest transcript. A dot matrix comparison between the PC23 and the three murine cDNAs isolated to date showed two regions of homology between PC23 and the

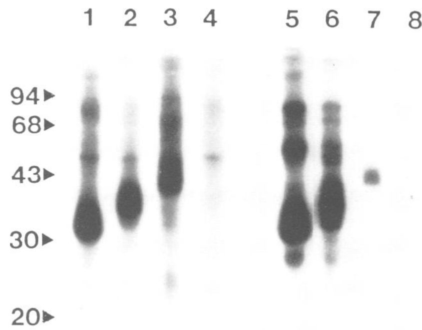

Fig. 3. IgG–agarose affinity precipitation and anti-CD32 immunoprecipitation of PC23 and TC9 encoded gene products. **Lanes 1–4** are IgG–agarose adsorptions, while **lanes 5–8** are CDw32 monoclonal antibody immunoprecipitations. Lane 1, TC9 transfected COS cells; lane 2, PC23 transfected COS cells; lane 3, HL-60 cells; lane 4, CD3 transfected COS cells. Lanes 5–8 are in the same order as lanes 1–4.

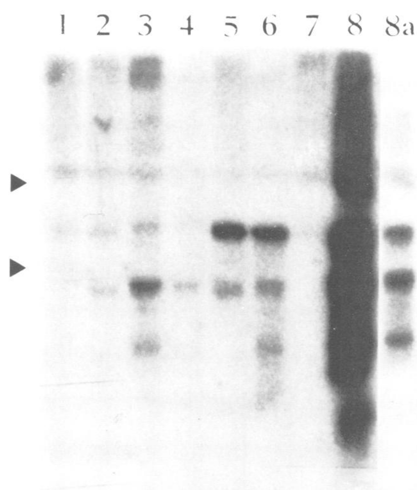

Fig. 4. RNA blot analysis of CDw32 transcripts. 20 μg of total RNA from different sources was fractionated on a 1% agarose gel, transferred to nylon, and hybridized to a PC23 probe. **Lane 1**, MOLT-4 (T leukemia); **lane 2**, PHA stimulated T cells (T blasts); **lane 3**, JY (EVB-transformed B cell line); **lane 4**, Raji (Burkitt's lymphoma); **lane 5**, HL-60 (promyelocytic leukemia); **lane 6**, U937 (histiocytic lymphoma); **lane 7**, HepG2 (hepatoblastoma) and **lane 8**, placenta. **Lane 8a** is a 10-fold shorter exposure of lane 8.

murine β_2 cDNA (Figure 6). The first region of homology represents sequences encoding the extracellular domains, while the second lies ~1 kb downstream in the 3′ noncoding region. The divergent segment between them begins a few bases 3′ to the junction between the transmembrane and cytoplasmic domains and ends at residue 1781 in the sequence Y_5GT_7CATG, presumably derived from the splice acceptor consensus $Y_{6-11}XCAG$ by a T insertion. An Alu family repeat element lies within the divergent region between residues 1393 and 1688. It thus appears that the inserted sequences are derived from an intron separating the transmembrane and intracytoplasmic domains in an ancestral form of the gene. Comparison of the human and murine peptide sequences shows that the homology is lost at the end of the transmembrane domain, in a region where the nucleotide sequence is still largely conserved, suggesting the existence of a selective pressure favoring the creation of a novel cytoplasmic domain. The molecular mass of the proposed mature polypeptide encoded by PC23 is 31 kd.

The TC9 sequence (Figure 7) corresponds to a fragment of a precursor RNA containing the complete coding sequence interrupted by an intron of ~1 kb, a transmembrane domain and a short intracellular domain terminating a few residues upstream from the 3′ end of the cDNA insert. As was similarly noted in the human CD7 cDNA sequence (Aruffo and Seed, 1987a), the presence of the intron disrupts a stem-and-loop structure predicted to occur in the mature TC9 transcript (Figure 7). The predicted secondary structure is thought to act as a barrier to efficient first strand synthesis in the cDNA reaction. A similar structure may not have the same significance for the PC23 transcript because several sequence changes substantially weaken the proposed stem but, as discussed below, a PC23-like cDNA isolated by a different approach (Hibbs *et al.*, 1988) shows evidence of inclusion of intron sequences at the same location. The TC9 intron contains an AATAAA sequence at position 1009, which may account for the smallest transcript observed in the RNA blot hybridization. Although there are few differences between the PC23 and TC9 coding sequences, the TC9 extracellular domain contains an additional site for N-linked glycosylation. The TC9 intracellular domain is quite short, perhaps artefactually so, because recent work on the genomic structure of CDw32-like genes has demonstrated the presence of an intron whose 5′ end lies two residues from the end of the open reading frame in the cDNA (J.Ravetch, personal communication). The splice donor in this case is not a particularly good match to the consensus and may not be used in all transcripts. Alternatively, the cDNA may be a fragment derived from a precursor bearing two introns. The mature polypeptide encoded by the cDNA is predicted to have a mass of 23.0 kd, 8 kd less than for the predicted PC23 polypeptide.

Recently the isolation and sequence analysis of a 1.4 kb cDNA related to PC23 has been reported (Stuart *et al.*, 1987; Hibbs *et al.*, 1988), as well as the sequence of an overlapping clone homologous to the 3′ end of the sequence reported here (Hibbs *et al.*, 1988). The sequences of the short cDNAs terminate just downstream from the AATAAA motif at position 1333. A 1.5 kb cDNA was also isolated which contained an insertion of 117 residues at the location of the intron in the TC9 clone (Hibbs *et al.*, 1988). The sequence of the insertion is highly homologous to the 5′ end of the TC9 intron and is bounded at the 3′ end by the exon sequences flanking the TC9 intron, suggesting that the insertion arose by utilization of an alternate splice donor in the interior of the intron.

Alignment of the two CDw32 receptors with the known murine forms (Figure 8) shows that both the signal sequence and the extracellular domain of the TC9 coding sequence are highly homologous to the comparable portions of the murine β forms; however, the intracellular domain is very short, like the murine α form. The signal sequence of the

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   1 CCCAAATGTCTCAGAATGTATGTCCCAGAAACCTGTGGCTGCTTCAACCATTGACAGTTTTGCTGCTGCTGGCTTCTGCAGACAGTCAAGCTGCAGCTCCCCCAAAGGCTGTGCTGAAAC
   1     M  S  Q  N  V  C  P  R  N  L  W  L  L  Q  P  L  T  V  L  L  L  L  A  S  A  D  S  Q  A  A  A  P  P  K  A  V  L  K  L

 121 TTGAGCCCCCGTGGATCAACGTGCTCCAGGAGGACTCTGTGACTCTGACATGCCAGGGGGCTCGCAGCCCTGAGAGCGACTCCATTCAGTGGTTCCACAATGGGAATCTCATTCCCACCC
  40  E  P  P  W  I  N  V  L  Q  E  D  S  V  T  L  T  C  Q  G  A  R  S  P  E  S  D  S  I  Q  W  F  H  N  G  N  L  I  P  T  H

 241 ACACGCAGCCCAGCTACAGGTTCAAGGCCAACAACAATGACAGCGGGGAGTACACGTGCCAGACTGGCCAGACCAGCCTCAGCGACCCTGTGCATCTGACTGTGCTTTCCGAATGGCTGG
  80  T  Q  P  S  Y  R  F  K  A  N  N  N  D  S  G  E  Y  T  C  Q  T  G  Q  T  S  L  S  D  P  V  H  L  T  V  L  S  E  W  L  V
                                       --CHO--

 361 TGCTCCAGACCCCTCACCTGGAGTTCCAGGAGGGAGAAACCATCATGCTGAGGTGCCACAGCTGGAAGGACAAGCCTCTGGTCAAGGTCACATTCTTCCAGAATGGAAAATCCCAGAAAT
 120  L  Q  T  P  H  L  E  F  Q  E  G  E  T  I  M  L  R  C  H  S  W  K  D  K  P  L  V  K  V  T  F  F  Q  N  G  K  S  Q  K  F

 481 TCTCCCGTTTGGATCCCACCTTCTCCATCCCACAAGCAAACCACAGTCACAGTGGTGATTACCACTGCACAGGAAACATAGGCTACACGCTGTTCTCATCCAAGCCTGTGACCATCACTG
 160  S  R  L  D  P  T  F  S  I  P  Q  A  N  H  S  H  S  G  D  Y  H  C  T  G  N  I  G  Y  T  L  F  S  S  K  P  V  T  I  T  V
                                          --CHO--

 601 TCCAAGTGCCCAGCATGGGCAGCTCTTCACCAATGGGGATCATTGTGGCTGTGGTCATTGCGACTGCTGTAGCAGCCATTGTTGCTGCTGTAGTGGCCTTGATCTACTGCAGGAAAAAGC
 200  Q  V  P  S  M  G  S  S  S  P  M  G  I  I  V  A  V  V  I  A  T  A  V  A  A  I  V  A  A  V  V  A  L  I  Y  C  R  K  K  R
                                  ------------------------------------TM-----------------------------------------

 721 GGATTTCAGCCAATTCCACTGATCCTGTGAAGGCTGCCCAATTTGAGCCACCTGGACGTCAAATGATTGCCATCAGAAAGAGACAACTTGAAGAAACCAACAATGACTATGAAACAGCTG
 240  I  S  A  N  S  T  D  P  V  K  A  A  Q  F  E  P  P  G  R  Q  M  I  A  I  R  K  R  Q  L  E  E  T  N  N  D  Y  E  T  A  D

 841 ACGGCGGCTACATGACTCTGAACCCCAGGGCACCTACTGACGATGATAAAAACATCTACCTGACTCTTCCTCCCAACGACCATGTCAACAGTAATAACTAAAGAGTAACGTTATGCCATG
 280  G  G  Y  M  T  L  N  P  R  A  P  T  D  D  D  K  N  I  Y  L  T  L  P  P  N  D  H  V  N  S  N  N  *

 961 TGGTCATACTCTCAGCTTGCTGAGTGGATGACAAAAGAGGGGAATTGTTAAAGGAAAATTTAAATGGAGACTGGAAAAATCCTGAGCAAACAAAACCACCTGGCCCTTAGAAATAGCTT

1081 TAACTTTGCTTAAACTACAAACACAAGCAAAACTTCACGGGGTCATACTACATACAAGCATAAGCAAAACTTAACTTGGATCATTTCTGGTAAATGCTTATGTTAGAAATAAGACAACCC

1201 CAGCCAATCACAAGCAGCCTACTAACATATAATTAGGTGACTAGGGACTTTCTAAGAAGATACCTACCCCCAAAAAACAATTATGTAATTGAAAACCAACCGATTGCCTTTATTTTGCTT

1321 CCACATTTTCCC[AATAAA]TACTTGCCTGTGACATTTTGCCACTGGAACACTAAACTTCATGAATTGCGCCTCAGATTTTTCCTTTAACATCTTTTTTTTTTTTGACAGAGTCTCAATCTG

1441 TTACCCAGGCTGGAGTGCAGTGGTGCTATCTTGGCTCACTGCAAACCCGCCTCCCAGGTTTAAGCGATTCTCATGCCTCAGCCTCCCAGTAGCTGGGATTAGAGGCATGTGCCATCATAC

1561 CCAGCTAATTTTTGTATTTTTTATTTTTTTTTTTTTAGTAGAGACAGGGTTTCGCAATGTTGGCCAGGCCGATCTCGAACTTCTGGCCTCTAGCGATCTGCCCGCCTCGGCCTCCCAAAGT

1681 GCTGGGATGACCAGCATCAGCCCCAATGTCCAGCCTCTTTAACATCTTCTTTCCTATGCCCTCTCTGTGGATCCCTACTGCTGGTTTCTGCCTTCTCCATGCTGAGAACAAAATCACCTA

1801 TTCACTGCTTATGCAGTCGGAAGCTCCAGAAGAACAAAGAGCCCAATTACCAGAACCACATTAAGTCTCCATTGTTTTGCCTTGGGATTTGAGAAGAGAATTAGAGAGGTGAGGATCTGG

1921 TATTTCCTGGACTAAATTCCCCTTGGGGAAGACGAAGGGATGCTGCAGTTCCAAAAGAGAAGGACTCTTCCAGAGTCATCTACCTGAGTCCCAAAGCTCCCTGTCCTGAAAGCCACAGAC

2041 AATATGGTCCCAAATGACTGACTGCACCTTCTGTGCCTCAGCCGTTCTTGACATCAAGAATCTTCTGTTCCACATCCACACAGCCAATACAATTAGTCAAACCACTGTTATTAACAGATG

2161 TAGCAACATGAGAAACGCTTATGTTACAGGTTACATGAGAGCAATCATGTAAGTCTATATGACTTCAGAAATGTTAAAATAGACTAACCTCTAACAACAAATTAAAAGTGATTGTTTCAA

2281 GGTGAAAAAA  2290
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Fig. 5. Nucleotide sequence of the PC23 cDNA. The nucleotide sequence and translated open reading frame are numbered at the left. Sites of potential N-linked glycosylation are depicted 'CHO' and the transmembrane region is underlined. An internal polyadenylation consensus sequence is shown boxed.

PC23 form is homologous to that of the murine α form, but its intracellular domain is rather long, more like the murine β form.

Discussion

Affinity cloning: a new method for cDNA isolation

In this report cDNA clones encoding human CDw32 Fc receptors (FcRII) were isolated by an extension of the method of expression library panning (Seed and Aruffo, 1987; Aruffo and Seed, 1987b). In the 'affinity cloning' procedure reported here, the ligand for a receptor acts as a bridge permitting adhesion of cells to dishes coated with antibody to the ligand. The ligand-mediated cloning process is similar to affinity chromatography, in which receptors are isolated by passage over solid supports to which ligand is attached. The present instance demonstrates that a relatively weak interaction ($K_a \sim 5 \times 10^5$ l/mol) suffices to allow isolation of a receptor by ligand adhesion. The weakness of the interaction also proved no impediment to characterization of iodinated receptor by adsorption to IgG agarose.

Fig. 6. Dot matrix comparison of the PC23 and murine β2 Fc receptor sequences. The locations of the domains of the two receptors are shown boxed. No homology is found between the signal peptides (S); good homology persists through the extracellular portion (EC) and transmembrane domain (TM), but a large insertion in the PC23 sequence disrupts the homology in the intracellular portion (IC). Further homology is encountered in the 3′ untranslated region (upper right corner).

Two forms of FcRII

The PC23 cDNA isolated by this method was an apparently full-length or near full-length clone of the largest mRNA species observed in RNA blot hybridizations. The existence of a polyadenylation signal in the cDNA at a location corresponding to the length observed for a smaller RNA suggests that alternate poly(A) site selection may be responsible for the two transcripts and the recent isolation of cDNAs corre-

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   1 CTGTGACTGCTGTGCTCTGGGCGCCACTCGCTCCAGGGAGTGATGGGAATCCTGTCATTCTTACCTGTCCTTGCCACTGAGAGTGACTGGGCTGACTGCAAGTCCCCCCAGCCTTGGGGT
   1                                            M  G  I  L  S  F  L  P  V  L  A  T  E  S  D  W  A  D  C  K  S  P  Q  P  W  G

 121 CATATGCTTCTGTGGACAGCTGTGCTATCCCTGGCTCCTGTTGCTGGGACACCTGCAGCTCCCCCAAAGGCTGTGCTGAAACTCGAGCCCCAGTGGATCAACGTGCTCCAGGAGGACTCT
  27 H  M  L  L  W  T  A  V  L  S  L  A  P  V  A  G  T  P  A  A  P  P  K  A  V  L  K  L  E  P  Q  W  I  N  V  L  Q  E  D  S

 241 GTGACTCTGACATGCCGGGGGACTCACAGCCCTGAGAGCGACTCCATTCAGTGGTTCCACAATGGGAATCTCATTCCCACCCACACGCAGCCCAGCTACAGGTTCAAGGCCAACAACAAT
  67 V  T  L  T  C  R  G  T  H  S  P  E  S  D  S  I  Q  W  F  H  N  G  N  L  I  P  T  H  T  Q  P  S  Y  R  F  K  A  N  N  N
                                                                                                                         ---
                                                                            <<<<
 361 GACAGCGGGGAGTACACGTGCCAGACTGGCCAGACCAGCCTCAGCGACCCTGTGCATCTGACTGTGCTTTCTGGTCAGTGGAGGAAGGCCCCAGGGTGGACCTGGGAGGGCCAGGACGGA
 107 D  S  G  E  Y  T  C  Q  T  G  Q  T  S  L  S  D  P  V  H  L  T  V  L  S
     CHO---

 481 TGAAATCTGCTTTCAGGCAGAGGTTTGCAGGAAAGGGGGGTGGCCTGCTTACTGGGAAGTATCGCTGTGAGTTGCCTCAGCACATATCAGTGGTTGTTTTTGCCTCAGTTCTGATTGAAC

 601 AGAAGAAGGTTTCAAGGCCAAAAACAGGCAGCCAAGTGTGAGAGAAGCAGAAGGAAATCCCTACTGCATAAAACCCATTTCCATTTTAATGGCAGAATTGAAAAGCACAGACCACAACTG

 721 AATCCTAGCCCTGGAAATGACTCACTATACAACATGATGAATTCATTTAACCCTTGAGTTTCCATTTCTTCACCTGCTCCGTGGGGCACTAACGCCTCCCTCAGAGGCTTCTGGTGAGAA

 841 TCAGTGTTTCCCTGCCCCCGCCCCGCCCTCCATGCCCCTTCTCCACGTTCTCACTGTGCTAGGTGCTCTTCTCTGTCTTTCTCTTCCACCAGCCTGTGGGAAACCTGAGATGAAAGTCGT

 961 GTCTTACCCATCTTTGTATTTCCAGCATCTGAAACTGGGCAGAGCTTAATAAATATTTTGCTGGAGAGGTTGATGATCTTACAAAGCTCCCATTGAAAGGTGGCTCTCTGTAAAGCAAAG

1081 TTACAATGAGATTGTGATGAACATTGTCCTTGTGGCTTTTCACTTAGTCCCCTCCCTTCACCTGAAGAGCAAATTTTCCTCAAAAGTACACAGCAAACGAATGACCCACTGGTGACACTG

1201 TTGCCTTTAGACCCTGCTGGAAAGAAGCTCCACATTTATTAACATTCCCGAAGTAAATTTATCAGGTAGCATTCATCAGGTAACATTTGTTGCACATTCATGACTTTTCTACTGTCCACA

1321 AAGGCATATGTCCTTATCATATGCGGACTCCTCGGTCACACTGGATTCTTCCTTCCCTCCTCGACATGGAAGAGATGGCATCTTAGGGTCTCTTGTGTTCTTCCTGCAGAGGCCTGTCGG

1441 GCAGGAAAAGGCTGCAGCTGCCTTCCTGGGAGAAGGAGGAGATGAGTGTATCCTGAACACCTATTATGTGCTAGGGGCTATTGTAGATACATGACACTATCATGCTCATTTTCACGAATG

1561 AGGAAACTGAGGCTCAGAAGACTTAAATTATTTGCCCAAGAGTTATAAATGACAGAGCCAGCATTAGAGTCCAGGACTGTCTGATTTCAGACCTAAGCTGTTCCCTCTGCACATCGTGTC
                                                                                                 <<<<      >>>>>>>>>
1681 CCACCAGTAAGGAAGATCTGGGTCTCAGAGCTGAGCCAAGACCTCCCGGGTCCTCTGCGGTTTTTTGTGTCTTTCAGAGTGGCTGGTGCTCCAGACCCCTCACCTGGAGTTCCAGGAGGG
 131                                                                                          E  W  L  V  L  Q  T  P  H  L  E  F  Q  E  G

1801 AGAAACCATCGTGCTGAGGTGCCACAGCTGGAAGGACAAGCCTCTGGTCAAGGTCACATTCTTCCAGAATGGAAAATCCAAGAAATTTTCCCGTTCGGATCCCAACTTCTCCATCCCACA
 146 E  T  I  V  L  R  C  H  S  W  K  D  K  P  L  V  K  V  T  F  F  Q  N  G  K  S  K  K  F  S  R  S  D  P  N  F  S  I  P  Q
                                                                                                                  ---CHO---

1921 AGCAAACCACAGTCACAGTGGTGATTACCACTGCACAGGAAACATAGGCTACACGCTGTACTCATCCAAGCCTGTGACCATCACTGTCCAAGCTCCCAGCTCTTCACCGATGGGGATCAT
 186 A  N  H  S  H  S  G  D  Y  H  C  T  G  N  I  G  Y  T  L  Y  S  S  K  P  V  T  I  T  V  Q  A  P  S  S  S  P  M  G  I  I
        ---CHO---                                                                                                 ----------

2041 TGTGGCTGTGGTCACTGGGATTGCTGTAGCGGCCATTGTTGCTGCTGTAGTGGCCTTGATCTACTGCAGGAAAAAGCGGATTTCAGGTTTGTAGCTCCTCCCGGTCCCTTTTGTTATCAG
 226 V  A  V  V  T  G  I  A  V  A  A  I  V  A  A  V  V  A  L  I  Y  C  R  K  K  R  I  S  G  L  *
     ----------------------TM----------------------------------------

2161 TTTCCACTTT
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Fig. 7. Nucleotide sequence of the TC9 cDNA. The nucleotide sequence and translated open reading frame are numbered at the left. Sites of potential N-linked glycosylation are depicted 'CHO' and the transmembrane region is underlined. The self-complementary region disrupted by the intron is denoted by < and > symbols above the sequence.

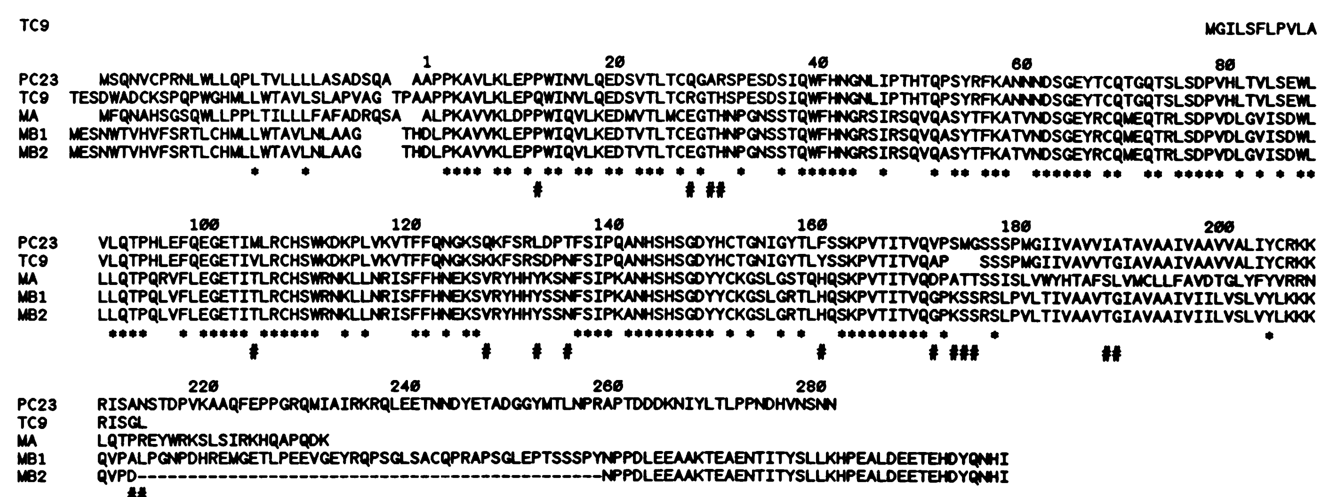

Fig. 8. Alignment of the murine IgG2b/1 and human CDw32 receptor sequences. The sequences shown are, from top to bottom, PC23, TC9, mouse α, mouse $\beta 1$ and mouse $\beta 2$. Asterisks denote identity between all five sequence, while # denotes differences between PC23 and TC9.

sponding to the shorter transcript (Stuart *et al.*, 1987; Hibbs *et al.*, 1988) supports this interpretation. The PC23 cDNA appears to contain an ancestral intron remnant which became part of the mRNA after mutation corrupted the splicing signals. An immediate effect of the intron inclusion must have been the alteration of the intracellular portion of the molecule.

The existence of an FcRII receptor of the same approximate site (40 kd) as the PC23 form has been well documented in the literature, but to date no unequivocal evidence

points to the existence of the short (34 kd) form encoded by TC9. Indeed the recent results of Ravetch and coworkers suggest that the mature form of this receptor may be larger, due to exonic coding sequences not present in the TC9 cDNA (J.Ravetch, personal communication). Unfortunately, the library source, a thyroid carcinoma biopsy specimen, does not shed much light on the cellular origin of this receptor. Tumor tissue is frequently invaded by diverse cell types including macrophages, granulocytes, lymphocytes and mast cells, any one of which might be the source of the TC9 receptor. Several groups have reported the existence of a 33 kd form of FcRII on granulocytes (Kulczycki, 1984; Vaughn *et al.*, 1985; Looney *et al.*, 1986) but this appears to be a proteolysis product of the 40 kd form. It remains possible that minority representation of the putative 34 kd form has gone unrecognized because it migrates with the same mobility as the proteolysis products.

Placenta proved to be an unusually rich source of CDw32 transcripts, as well as of at least one other macrophage specific RNA. Previous work has demonstrated that macrophages can be isolated in high yield from human placenta (Wood *et al.*, 1982; Uren and Boyle, 1985). Although placental IgG receptors have been implicated in the transplacental transport of maternal IgG (Matre and Tönder, 1982; Wood *et al.*, 1982; Matre *et al.*, 1984), the reported affinity of the trophoblast receptor is 20 times higher than the affinity measured here (Burton, 1985), making it unlikely that CDw32 is the trophoblast receptor.

Subtype binding specificity

Measurement of the affinity of the various IgG subtypes for the receptors isolated here have the rank order IgG1 > IgG2, IgG4 >> IgG3. This result is somewhat surprising, since various measurements on lymphocytes have yielded the order IgG1 ~ IgG3 >> IgG2, IgG4 (reviewed by Burton, 1985; Anderson and Looney, 1986); and Kulczycki (1984) has shown that IgG3 agarose (and IgG3 Fc fragment agarose) specifically binds granulocyte FcRII molecules. Several possibilities can be raised to reconcile the apparent discrepancy. (i) There may be other forms of low affinity receptor which have not yet been identified, such as the molecule recognized by monoclonal KB61. (ii) The receptors expressed on COS cells may differ in some important way from the receptors expressed on hematopoietic cells. (iii) The low binding affinity in COS cell experiments may be related to a specific inactivation of the IgG3 used. (iv) The IgG3 agarose binding result may not exclude a substantially lower affinity of receptor for IgG3.

Recent observations indirectly support the notion that IgG3 binding to mononuclear cells may be weaker than previously thought. Measurement of antibody dependent cellular cytotoxicity (ADCC) against target cells precoated with subtype specific antibodies has shown that IgG1 is substantially more effective than IgG3 in supporting ADCC mediated by peripheral blood mononuclear cells (Gergely *et al.*, 1986; Brüggemann *et al.*, 1987). Although receptor occupancy need not exclusively determine ADCC activity and the identities of all the types of receptors participating in ADCC are not known, these date provide support for the view that IgG1 binding to effector cells is substantially stronger than IgG3 binding under physiological conditions.

Relationship to murine receptors

Recently three distinct cDNAs were obtained for murine IgG2b/1-related receptors, α, $\beta 1$ and $\beta 2$. The latter two differ only by a differential splicing event which changes the intracellular domain. α and β extracellular domains are closely related, but not identical and the intracellular domains are divergent. In one study, the murine α clone was obtained by hybridization with an oligonucleotide prepared using N-terminal sequence information from purified IgG2b/1 receptor, and the two β clones were then obtained by cross-hybridization (Ravetch *et al.*, 1986); in two other reports, murine β clones were obtained by hybridization with oligonucleotide probes prepared from primary protein sequence data, or by detection of epitopes in an *E.coli* expression library using a polyclonal antiserum (Hibbs *et al.*, 1986; Lewis *et al.*, 1986). Expression of both of the β clones in COS cells gave cell surface expression detectable with an IgG2b/1 receptor-specific monoclonal antibody (Ravetch *et al.*, 1986). Thus all three clones probably participate in the expression of the 'IgG2b/1 receptor'.

The sequence of the PC23 clone is most closely related to the murine FcγR$\beta 2$ receptor sequence. The TC9 clone did not extend far enough into the 3′ untranslated region to affirm or deny its relationship to $\beta 2$, but the extracellular domain is, like that of PC23, clearly β-like. Both forms show striking divergence in the intracellular domain. This behavior is consistent with the pattern noted among the three murine cDNA species isolated thus far, in which the cytoplasmic domain is the least conserved portion of the molecule. The reason for this is not understood at present, but may be related to different intracellular programs initiated by cross-linking of IgG on receptor-bearing cells. Although the extracellular domains are highly homologous, the signal sequences of the two forms are very different, one apparently related to the murine α form signal peptide, and the other related to the β form. The utilization of different signal peptides for essentially the same extracellular domain implies that both α and β forms at one time lay in close proximity in the chromosome.

Materials and methods

cDNA libraries

RNA was prepared from cell lines or human biopsy tissue by the guanidinium thiocyanate method and cDNA was synthesized from the poly(A)$^+$ fraction as described (Aruffo and Seed, 1987b). The cDNA was inserted into the vector πH3M and transformed into *E.coli* MC1061/p3 (Aruffo and Seed, 1987b).

Recovery of clones by panning

The libraries were introduced into COS cells by spheroplast fusion as described (Seed and Aruffo, 1987). Two days after fusion the cells were detached from the plates by treatment with PBS containing 1 mM EDTA, treated either with purified antibodies at a concentration of 10 μg/ml, with a mixture of hybridoma supernatants, or with human serum, and distributed in dishes coated with affinity purified goat anti-human or sheep anti-mouse immunoglobulin antibodies as detailed previously (Seed and Aruffo, 1987). DNA was recovered from adherent cells by the Hirt procedure (Hirt, 1967), and transformed into *E.coli* for further rounds of transfection and panning.

DNA sequencing

The PC23 cDNA was inserted into M13 mp19 in both orientations and sequenced by a combination of deletion and oligonucleotide-directed dideoxy nucleotide sequencing techniques (Sanger *et al.*, 1977). Most of the deletions were made by cleavage of the cDNA clone with DNase I in the presence

of manganese ions (Frischauf *et al.*, 1980), followed by treatment with DNA polymerase I and cleavage with the enzymes *Xba*I and *Xho*I. Because the *Xho*I sites lie adjacent to the cDNA and just internal to the *Xba*I sites in the vector, the resulting fragments fall largely into three classes: those with flush and *Xho*I, *Xho*I and *Xba*I, and *Xba*I and flush ends. Only the flush/*Xho*I fragments can be cloned into M13 mp19 cut with *Sma*I and *Sal*I. In addition, a few specific deletions were made using restriction enzymes, and some regions of ambiguity were resolved by the use of oligonucleotide-primed synthesis. The TC9 clone was sequenced by oligonucleotide primed synthesis.

Transfections and radioimmunoprecipitations

COS cell transfections were carried out using DEAE dextran/chloroquine facilitation followed by DMSO shock (Sussman and Milman, 1984) as described elsewhere (Seed and Aruffo, 1987). Radioimmunoprecipitations were performed with a mixture of monoclonal anti-CDw32 antibodies following surface labelling with lactoperoxidase and $Na^{125}I$ (Clark and Einfeld, 1986; Seed and Aruffo, 1987). IgG affinity precipitations were carried out with commercial IgG agarose (Sigma); to extracts of surface iodinated cells ($2-4 \times 10^6$) in PBS/0.5% NP-40/0.05% sodium deoxycholate/0.05% SDS were added 25 μl of packed beads washed just prior to use. The extracts and beads were incubated together at 4°C for 1 h with constant mixing, then washed five times with PBS/1% NP-40, once with 20 mM Tris−HCl, pH 7.5, 50 mM NaCl, 0.5% NP-40 and eluted in sample buffer for electrophoresis (Laemmli, 1970).

Indirect immunofluorescence

COS cells transfected with the CDw32 clones were trypsinized 24 h after transfection and passaged into new plates to avoid nonspecific adhesion effects. For indirect immunofluorescence studies cells were incubated with antibodies at various concentrations in PBS containing 0.5 mM $CaCl_2$ and 1 mg/ml BSA, washed with 0.15 M NaCl, and treated in the same buffer with a fluorescein labelled affinity purified second antibody (Cappel, Malvern, PA). Results were scored by fluorescence microscopy.

Scatchard and displacement analyses

Purified human IgG subtypes were obtained from Calbiochem−Behring (La Jolla, CA) or as myeloma proteins kindly donated by Dr R.Kurrle, Behringwerke AG. Quantitative affinity measurements were performed with ^{125}I-labelled IgG1 (Calbiochem). 100 μl of 1 mg/ml IgG1 was incubated for 30 min with 1 mCi of $Na^{125}I$ and two Iodobeads (Pierce, Rockland, IL). Labelled protein was separated from unincorporated $^{125}I^-$ by passage over a column of Sephadex G25 (20−80 μ) equilibrated with PBS containing 5% nonfat milk and 0.2% NaN_3. Ligand was allowed to bind to transfected cells for > 15 min at 4°C in the same buffer, after which bound and free fractions were separated by centrifugation through 3:2 dibutyl:dioctyl phthalates and counted. Preliminary measurements showed that ligand binding equilibrium was reached by 8 min for both types of receptor under these conditions. Nonsaturable binding was measured in the presence of 2.5×10^{-4} M IgG and represented slightly more than 1% of the input counts. Subtype displacement experiments were carried out as above in the presence of iodinated IgG1 tracer at 2×10^{-8} M. Association constants were estimated from the half-saturation relation (Cheng and Prusoff, 1973); both displacing ligand and tracer were present in large excess over receptor concentration, allowing this approximation to be applied (Horovitz and Levitzki, 1987).

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Note added in proof

These sequence data have been submitted to the EMBL/GenBank sequence database under the accession number Y00857.

Recent experiments have demonstrated that both Fc receptors bind human IgD.