A cDNA Presumptively Coding for the α Subunit of the Receptor with High Affinity for Immunoglobulin E†

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ABSTRACT: Rat mast cells and a neoplastic analogue such as rat basophilic leukemia (RBL) cells have receptors that have exceptionally high affinity for immunoglobulin E (IgE). When aggregated, these receptors induce cellular degranulation. The α chain of the receptor contains the binding site for IgE; the function(s) of the noncovalently associated β and γ chains is (are) still undefined. Using a cDNA library constructed from the mRNA of RBL cells, we have isolated a cDNA clone whose sequence predicts a putative 23-residue signal peptide, followed by a sequence that accurately predicts the amino acid composition, the peptide molecular weight, and six peptide sequences (encompassing 59 residues or 26% of the total number) determined for the α chain by direct analysis. These findings provide strong evidence that the cDNA codes for the α chain, even though expression has not been unambiguously achieved. The sequence suggests that the α chain contains a 180-residue extracellular portion with two homologous domains of approximately 35 residues, a 20-residue transmembrane segment containing an aspartic acid, and a 27-residue cytoplasmic portion containing 9 basic amino acids. The sequence shows no homology with the low-affinity receptor for IgE from lymphocytes but over 30% homology with an Fcγ receptor.

The receptor $Fc_\epsilon RI$ with high affinity for immunoglobulin E (IgE)[1] is one of a family of proteins whose interactions with the Fc domains of immunoglobulins transforms the reaction of antibody with antigen into a physiologically important event. Unlike the low-affinity receptor for IgE, $Fc_\epsilon RI$ is found exclusively on mast cells, basophils, and related cells. Aggregation of this receptor triggers both the release of preformed mediators such as histamine and serotonin and the synthesis of leukotrienes (Metzger et al., 1986). The most thoroughly characterized $Fc_\epsilon RI$ is that of the rat basophilic leukemia (RBL) cell line. It consists of three different types of subunits: one 40–50-kDa glycoprotein α chain, which contains the binding site for IgE (Conrad et al., 1976; Kulczycki et al., 1976), a single 33-kDa β chain (Holowka et al., 1980; Holowka & Metzger, 1982; Perez-Montfort et al., 1983a), and two 7–9-kDa disulfide-linked γ chains (Perez-Montfort et al., 1983a; Alcaraz et al., 1987). This paper describes the isolation of a cDNA clone that codes for the α subunit of the receptor. Our basic approach was to screen a cDNA library with an oligonucleotide probe on the basis of a sequence derived by direct analysis of the α chain; because the amino terminus of the α chain is blocked, peptides were isolated in order to obtain the necessary sequence.

Experimental Procedures

Peptide Sequencing of the α Chain. Electroeluted α chains were prepared (Alcaraz et al., 1987) in 6% yield. When they were checked for purity by silver staining, no contaminants were visible. A total of 0.5–1 nmol of the α chain was digested with trypsin in 2 M urea at an enzyme to protein ratio of 1:25 at 37 °C for 24 h. The digest was fractionated with a 0–60% acetonitrile gradient on an HPLC C18 reverse-phase Vydac column, and the separated peptides were applied to a gas-phase microsequencer apparatus (Applied Biosystem Model 4708 on line with HPLC Applied Biosystem 120).

cDNA Cloning, Screening, and Sequencing. RNA was extracted from RBL cells by the guanidinium isothiocyanate procedure (Chirgwin et al., 1979); poly(A+) RNA was isolated and size fractionated by velocity sedimentation on sucrose gradients (Portnoy et al., 1986). The size fraction corresponding to 14S was subsequently used, as it corresponded to the size class of RNA identified to code for IgE binding activity on *Xenopus* oocytes (Liu & Orida, 1984; Pure et al., 1984) and it corresponded closely to the size of RNA coding for the murine Fcγ receptor (Ravetch et al., 1986). cDNA synthesis was performed as previously described (Kochan et al., 1986), with the exception that methylmercury hydroxide was omitted during first strand synthesis, DNA *pol*I was substituted for Klenow in the second strand reaction, and S1 nuclease instead of Mung bean nuclease was used to blunt end the cDNA molecules. The resulting cDNA molecules were dC tailed, size fractionated, annealed to dG-tailed puc-9 (Pharmacia), and used to transform *Escherichia coli* strain MC1061. A total of 32 000 independent clones were generated, and these were stored on nitrocellulose filters impregnated with 20% glycerol at −20 °C. Sixteen oligonucleotides having the composite sequence 5′TTGAA$^{A}_{G}$GCAAT$^{G}_{C}$ACIIICT-T$^{A}_{G}$TA$^{A}_{G}$TAGAT3′ corresponding to the complementary strand coding for peptide 1-1 (Table I) were synthesized (Beaucage & Caruthers, 1981). The rationale for codon selection was based on that of Lathe (1985). Three 5′-deoxyinosines were inserted in the position coding for the unknown internal amino acid residue (Results). The oligonucleotides were end labeled

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[1] Abbreviations: IgE, immunoglobulin E; IgG, immunoglobulin G; RBL, rat basophilic leukemia; $NaDodSO_4$, sodium dodecyl sulfate; kDa, kilodalton; bp, base pairs; HPLC, high-pressure liquid chromatography; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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Table I: Protein Sequence Obtained from Tryptic Peptides of the α Chain

expt[a]	peptides	sequence	correspondence with deduced sequence	yield (%)[b]
1	1-1	?-I-Y-Y-K-?-V-I-A-F-K[c]	141–151	1
2	2-1	V-I-Y-Y-K-D-D-I-A-F-K	141–151	18.8
2	2-2[d]	I-L-T-G-D-K-V-T-L-I	39–48	12.8
2	2-3[d]	W-I-H-N-D-S-I-S-N-V-K	62–72	10.2
2	2-4[d]	Y-S-Y-D-S-N	152–157	5.4
2	2-5	S-S-H-W-V-I-V-S-A-T-I-Q-D-S-G-K	73–88	55.1
2	2-6	Y-I-C-Q-K	89–93	22.5

[a] In experiment 2 the α chain was reduced and carboxymethylated prior to digestion. [b] The yield was calculated as follows: (average picomoles of residue in the two first cycles of sequence × 100)/(picomoles of α chain subjected to tryptic cleavage). The yield reflects the successive loss of material due to ethanol precipitation, incomplete digestion, separation of peptides, collection of peptides, and sequencing. The sequenced residues were compared to standards for quantitation. [c] The one-letter abbreviations for amino acids are used. [d] Peptides 2-2, 2-3, and 2-4 were collected in a single peak, and the mixture was sequenced. Each cycle of the sequence gave residues in a similar yield so that it was impossible to assign one residue to one particular peptide. For clarity we have assigned the residues to the peptide predicted by the cDNA sequence.

with ^{32}P by use of T4 polynucleotide kinase and [γ-^{32}P]ATP (Maniatis et al., 1982) and used to screen our cDNA library. Six clones were identified by oligonucleotide hybridization in a 0.9 M NaCl buffer containing 10× Denhardt's solution (Maniatis, 1982), 0.1% $NaDodSO_4$, 90 mM Tris, pH 8.3, 6 mM EDTA, and 50 μg/mL *E. coli* DNA at a temperature of 45 °C. Washes were as in Maniatis (1982). These all corresponded to the same cDNA by Southern blot hybridization and restriction enzyme mapping. The DNA sequence was determined (Maxam & Gilbert, 1977) for both strands of three cDNA clones (clone 1, nucleotides 42–517; clone 2, nucleotides 89–1066; clone 4, nucleotides 1–698), and no discrepancies in sequence were observed for all regions of overlap.

Results

Isolation and Sequencing of cDNA. An initial peptide having the apparent sequence ?IYYK?VIAFK was obtained in low yield (Table I). The first and sixth residue could not be clearly identified, and there was some uncertainty at positions 5, 7, 9, and 10. Nevertheless, we opted to construct a probe corresponding to the complementary strand of the presumptive mRNA for the above decapeptide beginning with isoleucine (Experimental Procedures). A cDNA library (Experimental Procedures) was screened, and six positive clones were identified. They cross-hybridized, and three were chosen for sequence analysis. An open reading frame of 250 amino acids was found, beginning with an ATG at nucleotide 27 and ending at nucleotide 776 (Figure 1). The predicted signal peptidase cleavage site follows either Thr-23 (von Heijne, 1983) or Ala-24 (von Heijne, 1986). Because, as noted, the N-terminus of the α subunit is blocked (Kannelopoulos et al., 1980; M. Bond, personal communication, reconfirmed in this study), we could not determine the amino terminus directly by sequencing the intact chain.

During the course of the cloning and sequencing of the cDNA, additional peptides were isolated from further tryptic digests (Table I).

Comparison of Predicted Sequence with Direct Analysis. The cDNA predicts an amino acid sequence that corresponds

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TGCAGTTAGCACCGAAGGCACGGGCA  ATG GGT ACC GGA GGA TCT GCC CGG CTG TGC CTA 59
                            MET Gly Thr Gly Gly Ser Ala Arg Leu Cys Leu
                                                                    10

GCA TTA GTG CTC ATA TCT CTG GGT GTC ATG CTA ACA↓GCC ACT CAG AAA TCT GTA 113
Ala Leu Val Leu Ile Ser Leu Gly Val MET Leu Thr Ala Thr Gln Lys Ser Val
                                  20

GTG TCC TTG GAC CCA CCG TGG ATT AGA ATA CTT ACA GGA GAT AAA GTG ACT CTT 167
Val Ser Leu Asp Pro Pro Trp Ile Arg Ile Leu Thr Gly Asp Lys Val Thr Leu
 30                                  40

ATA TGC AAT GGG AAC AAT TCC TCT CAA ATG AAC TCT ACT AAA TGG ATC CAC AAT 221
Ile Cys Asn Gly Asn Asn Ser Ser Gln MET Asn Ser Thr Lys Trp Ile His Asn
        50                                  60

GAT AGC ATC TCT AAT GTG AAA TCG TCA CAT TGG GTC ATT GTG AGT GCC ACC ATT 275
Asp Ser Ile Ser Asn Val Lys Ser Ser His Trp Val Ile Val Ser Ala Thr Ile
                70                                  80

CAA GAC AGT GGA AAA TAC ATA TGT CAG AAG CAA GGA TTT TAT AAG AGC AAA CCT 329
Gln Asp Ser Gly Lys Tyr Ile Cys Gln Lys Gln Gly Phe Tyr Lys Ser Lys Pro
                    90                                  100

GTG TAC TTG AAC GTG ATG CAA GAG TGG CTG CTG CTC CAA TCT TCT GCT GAC GTG 383
Val Tyr Leu Asn Val MET Gln Glu Trp Leu Leu Leu Gln Ser Ser Ala Asp Val
                        110

GTC TTA GAC AAT GGA TCC TTT GAC ATC AGA TGC CGT AGC TGG AAG AAA TGG AAA 437
Val Leu Asp Asn Gly Ser Phe Asp Ile Arg Cys Arg Ser Trp Lys Lys Trp Lys
120                                 130

GTC CAC AAG GTG ATC TAC TAC AAG GAC GAC ATT GCT TTC AAG TAC TCT TAT GAC 491
Val His Lys Val Ile Tyr Tyr Lys Asp Asp Ile Ala Phe Lys Tyr Ser Tyr Asp
        140                                 150

AGC AAC AAC ATC TCC ATT AGA AAG GCC ACA TTT AAT GAC AGT GGC AGC TAC CAC 545
Ser Asn Asn Ile Ser Ile Arg Lys Ala Thr Phe Asn Asp Ser Gly Ser Tyr His
                160                                 170

TGC ACA GGC TAT TTG AAC AAG GTT GAA TGT AAA TCT GAT AAA TTC AGT ATT GCT 599
Cys Thr Gly Tyr Leu Asn Lys Val Glu Cys Lys Ser Asp Lys Phe Ser Ile Ala
                    180                                 190

GTA GTA AAA GAT TAC ACA ATT GAG TAT CGT TGG CTA CAA CTC ATT TTC CCA TCA 653
Val Val Lys Asp Tyr Thr Ile Glu Tyr Arg Trp Leu Gln Leu Ile Phe Pro Ser
                        200

TTG GCG GTG ATT CTG TTT GCT GTG GAT ACT GGG TTA TGG TTC TCA ACC CAC AAA 707
Leu Ala Val Ile Leu Phe Ala Val Asp Thr Gly Leu Trp Phe Ser Thr His Lys
210                                 220

CAG TTC GAA TCC ATC TTG AAG ATT CAG AAC ACT GGA AAA GGC AAA AAA AAA AGG 761
Gln Phe Glu Ser Ile Leu Lys Ile Gln Asn Thr Gly Lys Gly Lys Lys Lys Arg
        230                                 240

TTG AAA CCT AAC TCT  TAACCAAGGTATATAAAGGAACTAATGTCATCGCTTAAGAGACAATTCTT 826
Leu Lys Pro Asn Ser
                250

AACAATTATTTCCCACAGTATCTTCAATAGCCTTTCAACTGTCAAAGGACACTCATGTTATCCATAGAAAT 897

GTCTGTACCCCAGGAATTGCATAAATGCTTCATTAAACCAACAGCAGCTGGTTAAGTGACATGCAATAAAT 968

AACAAATACTCAATAAACACTGGTTTAATGAAGCATTT 1006
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FIGURE 1: Nucleotide sequence and predicted amino acid sequence of the rat $Fc_\epsilon RI(\alpha)$. The numbers at the end of each line and below the line refer to nucleotide and amino acid positions, respectively. Amino acids 1–23 represent the predicted signal sequence, and the hydrophobic core is underlined. The arrow indicates the preferred signal peptidase cleavage as determined by the algorithm of von Heijne (1986). Asparagine residues of the predicted N-linked glycosylation sites (Asn-X-Ser/Thr) are boxed. The hydrophobic stretch encompassing amino acids 204–223 suggestive of a transmembrane domain is also underlined.

extremely well with all of the known properties of the isolated α subunit. Peptide 2-1 was isolated in almost 20% yield and has a sequence corresponding closely to the peptide on which the initial probe was based. The unknown first residue proved to be valine; the unknown sixth residue, aspartic acid; and the ambiguous valine at position 7, another aspartic acid. Since the latter two residues have codons that differ only by a substitution of an A for a T in position 2, it is not surprising that adequate hybridization of the probe occurred despite this one base difference. Peptides 2-2, 2-3, and 2-4, isolated in 5–13% yields, eluted together but gave well-defined residues at each position in the sequence analysis. The combined data are consistent with the deduced sequence for residues 39–48, 62–72, and 152–157, respectively. Peptide 2-5 was obtained with a 55% yield, and its sequence corresponds perfectly with the deduced residues 73–88. Finally, the pentapeptide 2-6 obtained in 22% yield corresponds to the sequence for positions 89–93. Significantly, no peptides were recovered whose sequence was incompatible with the sequence predicted by the cDNA we isolated.

In addition, the predicted amino acid composition agrees well with the composition of the α subunit previously reported (Table II). Particularly striking is the correspondence of the eight tryptophans predicted by the cDNA with the minimal

Table II: Comparison of Composition of the α Subunit Deduced from cDNA and Determined by Direct Analysis

method	residue								
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met
deduced from cDNA	28	11	27	14	5	10	8	17	2
direct analysis[a]									
A[b]	28 ± 1	12 ± 1	23 ± 1	17 ± 1	6 ± 1	14 ± 1	10 ± 1	17 ± 1	2 ± 0
B[c]	23 ± 3	10 ± 2	20 ± 4	21 ± 3	7 ± 3	19 ± 4[d]	11 ± 3	14 ± 2	3 ± 1

method	residue								
	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys	Trp
deduced from cDNA	19	16	11	9	5	26	6	5	8
direct analysis									
A	16 ± 0	17 ± 0	10 ± 0	9 ± 0	4 ± 0	22 ± 2	6 ± 1	5 ± 1	≥2
B	15 ± 2	21 ± 5	10 ± 4	10 ± 3	5 ± 1	17 ± 4	8 ± 1	4 ± 1	≥8[e]

[a] Based on the experimental values of the mole percent of each amino acid and assuming that the total number of residues (subtracting the tryptophan) is 219 as suggested by the cDNA. [b] Kanellopoulos et al. (1980). [c] Alcaraz et al. (1987). [d] The high glycine content is likely related to the buffer used in the final stage of purification (Alcaraz et al., 1987). [e] From studies of biosynthetic incorporation (Alcaraz et al., 1987).

number of eight predicted from incorporation studies (Alcaraz et al., 1987).

The molecular weight predicted for the processed protein (26 104) is close to the value of 27 300 ± 300 measured by chromatography on Sepharose 6B in 6 M guanidine hydrochloride after treatment of the α subunit with endoglycosidase (Kumar & Metzger, 1982) and to the value of 27 000 estimated from similar chromatography of the intact glycoprotein [assuming a carbohydrate content of ~30% (Kanellopoulos et al., 1980)] (Kumar & Metzger, 1982). Finally, it agrees with the estimate made for the size of the solubilized IgE binding component by radiation inactivation, although the cell-bound target size was somewhat higher (Fewtrell et al., 1981). The α chain is thought to contain both O-linked and N-linked carbohydrates (Goetze et al., 1981; Hempstead et al., 1981a). The cDNA predicts seven residues to which N-linked sugars could potentially be attached.

Northern Blot Studies. Poly(A+) mRNA purified from RBL cells and the rat lymphoma cell line W/Fu(C58NT)D("NTD") (Geering et al., 1966) were separated on a formaldehyde agarose gel (Lehrach et al., 1977), transferred to nitrocellulose, and hybridized under stringent conditions to a nick-translated *Sty*I fragment (that codes for all but the amino-terminal eight amino acids of the processed peptide). As can be seen in Figure 2, mRNA from RBL cells reacts with the probe. There is a major species of 1.3 kb and an additional species around 2.9 kb. The relationship between these two transcripts is currently under investigation. The lymphoma cells, which do not express the high-affinity receptor for IgE (Isersky et al., 1978) but which bind aggregated mouse IgG (J. Rivera, unpublished observations), yielded no hybridizable mRNA (Figure 2).

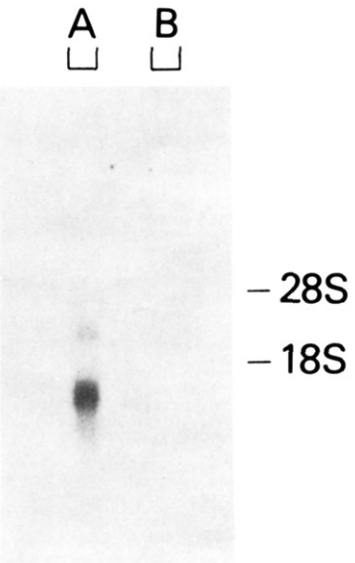

FIGURE 2: Northen blot analysis. Poly(A+) RNA purified from RBL $Fc_\epsilon R$ positive cells (lane A) and rat lymphoma $Fc_\epsilon R$ negative cells (lane B) were hybridized under stringent conditions with a nick-translated *Sty*I fragment (nucleotides 119–781). The ribosomal RNA markers are indicated on the right.

Discussion

Topology of the α Subunit. Direct experimental assessment is necessary to confirm the transmembrane topology suggested by the sequence. Previous data on the disposition of the α chain have not been definitive. Data inconsistent with a transmembrane disposition were (1) the failure of α to become labeled with an intramembrane probe that successfully labeled the β and γ chains (Holowka et al., 1982; Perez-Montfort et al., 1983a) and (2) the failure of oxidative iodination to modify the α subunit on inside-out vesicles (Isersky et al., 1982; Holowka & Baird, 1984). The current sequence easily explains the latter results since the putative cytoplasmic tail contains no tyrosine (Figure 1). On the other hand, there is persuasive evidence that the α subunit interacts with lipids and detergents (Alcaraz et al., 1984; Kinet et al., 1985a,b), which would be consistent with a transmembrane configuration. Furthermore, we have learned that a particular monoclonal anti-α reacts with inside-out membrane vesicles but not with the intact cells from which the vesicles were derived (B. Baird and D. Holowka, personal communication). Finally, although we have never observed this with intact RBL cells (Perez-Montfort et al., 1983b; Quarto & Metzger, 1986), Hempstead et al. (1981b, 1983) have reported phosphorylated serine and threonine residues on the α chains of receptor from rat peritoneal mast cells. The putative cytoplasmic tail sequence contains potential sites for such modification.

Homology with Other Proteins. The α subunit is clearly a member of the immunoglobulin supergene family, as the Protein Identification Resource program RELATE identified numerous homologies to immunoglobulin genes and their family members. It is most homologous to the murine $Fc\gamma R(\alpha)$ gene (Ravetch et al., 1986) and less to the related $Fc\gamma R(\beta)$ genes (Ravetch et al., 1986; Lewis et al., 1986; Hibbs et al., 1986). Overall there is 32% identity between the amino acid sequences encoded by the two genes (Figure 3), and use of the ALIGN program indicates that the homologies are more than 23 standard deviations above a randomly generated score. The homology is even greater at the nucleic acid level—about 49% within the coding regions for the two genes. The $Fc\gamma R(\alpha)$ sequence suggests two homologous domains within the ex-

Table III: Comparison of Genes for IgE Receptor/Binding Factors

protein	cellular distribution	properties of predicted protein: peptide M_r ($\times 10^{-3}$)	properties of predicted protein: other	reference
α subunit of high-affinity receptor	mast cells, RBL cells	26	homology to FcγR(α)	this study
low-affinity receptor	B(?T) lymphocytes	36	homology to asialoglycoprotein receptor; "inside-out" topology	Kikutani et al. (1986); Ludin et al. (1987); Ikuta et al. (1987)
IgE binding	T lymphocytes	62	contains retrovial polymerase sequence	Martens et al. (1985)
IgE binding	RBL, other?	31	high content of Pro, Gly, and Ala; repetitive sequences of YPGXXXPGA	Liu et al. (1985)

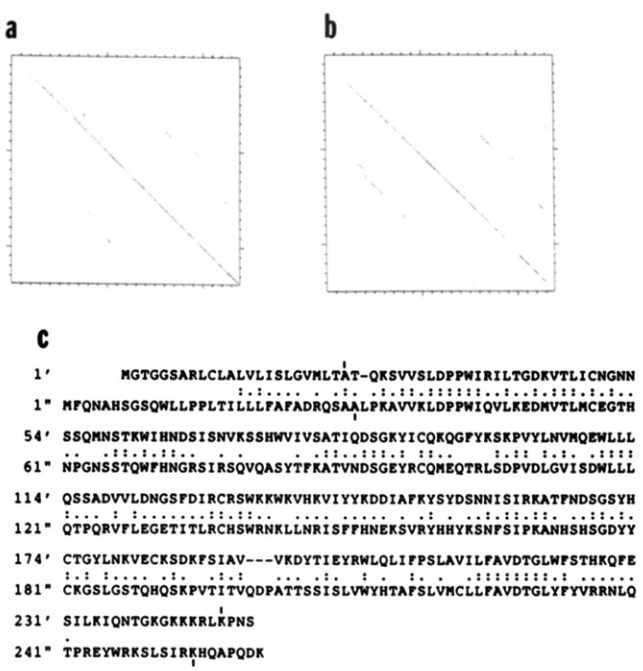

FIGURE 3: Amino acid sequence comparison of Fc$_\epsilon$RI(α) and FcγR(α). (a) Self-comparison of the Fc$_\epsilon$RI(α) amino acid sequence using the DOTMATRIX program of the Protein Identification Resource. A diagonal line of identity is seen, as well as two smaller regions of internally repeated sequences. (b) DOTMATRIX comparison of Fc$_\epsilon$RI(α) (abscissa) and FcγR(α) (ordinate). An off-center diagnoal line is seen, demonstrating a high level of homology between the two sequences. In addition, an off-diagonal line is present, demonstrating a high level of homology between domains 1 and 2 of the Fc$_\epsilon$RI(α) and FcγR(α). The level of homology between the different domains in the two receptors is higher than the homology within the two domains of each individual receptor. (c) The program FASTP was used to align Fc$_\epsilon$RI(α) (1'–250') and FcγR(α) (1"–261"). Gaps have been introduced to optimize the alignment. Two dots indicate identity, and one dot indicates a conservative mutation.

tracellular domain (Ravetch et al., 1986). Similarly, for Fc$_\epsilon$RI(α) the sequences encompassing amino acids 65–102 and 148–182 show 26% identity. It is interesting that the two domains present in both Fc$_\epsilon$RI(α) (Figure 3a) and FcγR(α) show slightly stronger homology between the two genes (Figure 3b) (34% identity in each case) than within each gene. This suggests that the genes have a common origin and that there has been more intragenic divergence to meet specific requirements than intergenic divergence. The greatest homology between the two α chains is localized in two segments: in the N-terminal region where 10 of 14 amino acids are identical and within the transmembrane segment where 8 amino acids surrounding the common aspartic acid residue are identical (Figure 3c). The potential functional significance of these highly conserved structures deserves study.

On the other hand, the gene for the α subunit shows no homology with the recently described genes that code for several other proteins that bind IgE (Table III).

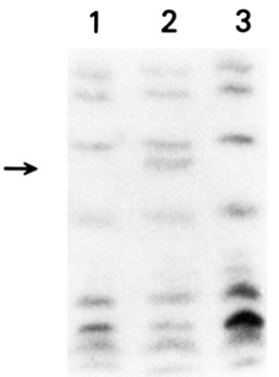

FIGURE 4: *E. coli* expression of a fragment from the cDNA coding region of the α chain. *E. coli* transformants (Crowl et al., 1985) containing the insert in the wrong orientation (lane 1) and in the correct orientation (lane 2) and *E. coli* without an insert (lane 3) were extracted with sample buffer containing NaDodSO$_4$; the proteins were separated on a 12.5% NaDodSO$_4$ gel and transferred electrophoretically onto nitrocellulose paper. The paper was incubated overnight at 4 °C with an IgG fraction of rabbit polyclonal anti-α, washed, and then reacted with ^{125}I-labeled protein A. It was finally subjected to autoradiography. The arrow on the left indicates the position of 18 kDa, based on molecular weight standards blotted simultaneously. Lane 2 shows an extra band in that position. The multiple bands seen in all samples are likely due to "natural" antibodies to *E. coli* proteins present in rabbits since they are regularly seen with preimmune sera also. The figure shows an enlargement of the lower half of the radioautograph of the gel.

Expression. Several approaches were tried to express the cDNA as a protein reactive with antibodies to the α subunit or with IgE, but all were unsuccessful or inconclusive. In vitro transcription and translation of the full-length cDNA generated an ~26-kDa protein that corresponds to the unprocessed peptide. The failure to immunoprecipitate the 26-kDa or a partly modified 45-kDa peptide, with IgE and anti-IgE or with different antibodies, could be due to several factors. The absence of adequate posttranslational modifications and the absence of the other subunits in this in vitro system might prevent proper folding of the α chain. Expression has been achieved in *E. coli* of an 18-kDa protein corresponding to the *Hin*fI fragment of nucleotides 163–662 of the cDNA (Figure 4). This protein reacted with a polyclonal anti-α antibody (Isersky et al., 1978) in a Western blot assay. However, in a similar assay, this antibody reacts with several proteins in an unfractionated detergent extract from RBL cells. We have too little of the antibody to make fractionation of it by affinity techniques practical. Therefore, this experiment cannot provide unambiguous evidence that the expressed protein corresponds to the α chain. Transcripts specific for the α gene were detected after transfection of COS cells (SV40 transformants of African green monkey cells), but α chains were not detected either on the surface or inside COS cells. This could have been due to a variety of reasons (Bole et al., 1986; Gething et al., 1986; Blobel, 1985; Kaufman & Murtha, 1987).

CONCLUSIONS

Procedures were previously developed (Kanellopoulos et al., 1979) by which the receptor with high affinity for monomeric IgE could be isolated uncontaminated with significant amounts of either other immunoglobulin-binding receptors on these cells (Froese, 1980; Segal et al., 1980) or other membrane proteins. Detailed studies of the molecular weight (Fewtrell et al., 1981; Kumar & Metzger, 1982) and of the amino acid composition both by direct analysis (Kanellopoulos et al., 1980; Alcaraz et al., 1987) and by biosynthetic incorporation (Alcaraz et al., 1987) have been used to develop structural criteria that define the IgE binding α subunit of the receptor. In this paper these characteristics have been extended to include sequence data encompassing over 25% of the protein's total residues. We have now isolated a cDNA that correctly predicts *all* of the peptide sequences (distributed over approximately half of the total sequence), the critical elements of the amino acid composition, and the peptide molecular weight. A variety of other, less decisive structural features are also explicable by the cDNA sequence (Results). Together these results leave little room for doubt that the cDNA we have isolated codes for the α chain of the receptor.

In order to utilize this cDNA to develop further information about the structure and function of the receptor, it will of course be necessary to express this gene. However, there is a distinct possibility that full expression may not be feasible unless the three types of subunits are cosynthesized (McPhaul & Berg, 1986; Bole et al., 1986). For example, even though the α subunit continues to show IgE binding activity after dissociation of the β and γ chains (Kinet et al., 1985a), the latter might be required to cause the α subunit to be folded correctly in the first place. It may be relevant that when the β and γ chains are dissociated from the α subunit, the latter fails to incorporate into liposomes (Rivnay & Metzger, 1982). Therefore, we are proceeding directly to isolate the cDNAs that code for the β and γ chains. For these chains also, we have extensive structural data by which to judge whether a putative cDNA is the correct one (Alcaraz et al., 1987; Holowka & Metzger, 1982). Cotransfection with the cDNAs for all three of the subunits should provide a more adequate strategy for obtaining expression of the receptor's structure and function. It is interesting to note that the gene to which the α subunit of the receptor for IgE is most closely related—the "α" protein of the receptor that binds IgG (above)—has also not yet been expressed (Ravetch et al., 1986). Possibly this receptor also contains additional subunits (that have so far eluded detection) whose cosynthesis may be necessary to achieve adequate expression.

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REFERENCES

Alcaraz, G., Kinet, J.-P., Kumar, N., Wank, S. A., & Metzger, H. (1984) *J. Biol. Chem. 259*, 14922–14927.

Alcaraz, G., Kinet, J.-P., Liu, T. Y., & Metzger, H. (1987) *Biochemistry 26*, 2569–2575.

Beaucage, S. L., & Caruthers, M. H. (1981) *Tetrahedron Lett. 22*, 1859–1862.

Blobel, G. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8527–8529.

Bole, D. G., Hendershot, L. M., & Kearney, J. F. (1986) *J. Cell Biol. 102*, 1558–1566.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Conrad, D. H., Berczi, I., & Froese, A. (1976) *Immunochemistry 13*, 329–332.

Crowl, R., Seamans, C., LoMedico, P., & McAndrew, S. (1985) *Gene 38*, 31–38.

Fewtrell, C., Kempner, E., Poy, G., & Metzger, H. (1981) *Biochemistry 20*, 6589–6594.

Froese, A. J. (1986) *J. Immunol. 125*, 981–987.

Geering, G., Old, L. J., & Boyse, E. A. (1966) *J. Exp. Med. 124*, 753–772.

Gething, M.-J., McGammon, K., & Sambrook, J. (1986) *Cell (Cambridge, Mass.) 46*, 939–950.

Goetze, A., Kannelopoulos, J., Rice, D., & Metzger, H. (1981) *Biochemistry 20*, 6314–6349.

Hempstead, B. L., Parker, C. W., & Kulczycki, A., Jr. (1981a) *J. Biol. Chem. 256*, 10717–10723.

Hempstead, B. L., Kulczucki, A., Jr., & Parker, C. W. (1981b) *Biochem. Biophys. Res. Commun. 98*, 815–822.

Hempstead, B. L., Parker, C. W., & Kulczycki, A., Jr. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3050–3053.

Hibbs, M. L., Walker, I. D., Kirszbaum, L., Pietersz, G. A., Deacon, N. J., Chambers, G. W., McKenzie, I. F. C., & Hogarth, P. M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6980–6984.

Holowka, D., & Metzger, H. (1982) *Mol. Immunol. 19*, 219–227.

Holowka, D., & Baird, B. (1984) *J. Biol. Chem. 259*, 3720–3728.

Holowka, D., Hartmann, H., Kannelopoulos, J., & Metzger, H. (1980) *J. Recept. Res. 1*, 41–68.

Ikuta, K., Takami, M., Kim, C. W., Honjo, T., Miyoshi, T., Tagaya, Y., Kawabe, T., & Yodoi, J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 819–823.

Isersky, C., Taurog, J. D., Poy, G., & Metzger, H. (1978) *J. Immunol. 121*, 549–558.

Isersky, C., Rivera, J., Triche, T., & Metzger, H. (1982) *Mol. Immunol. 19*, 925–941.

Kannelopoulos, J., Rossi, G., & Metzger, H. (1979) *J. Biol. Chem. 254*, 7691–7697.

Kannelopoulos, J., Liu, T. Y., Poy, G., & Metzger, H. (1980) *J. Biol. Chem. 255*, 9060–9066.

Kaufman, R. J., & Murtha, P. (1987) *Mol. Cell. Biol. 7*, 1568–1571.

Kikutani, H., Inui, S., Sato, R., Barsumian, E. L., Owaki, H., Yamasaki, K., Kaisho, T., Uchiboyashi, N., Hardy, R. R., Hirano, T., Tsurasawa, S., Sakiyama, F., Suemura, M., & Kishimoto, T. (1986) *Cell (Cambridge, Mass.) 47*, 657–665.

Kinet, J.-P., Alcaraz, G., Leonard, A., Wank, S., & Metzger, H. (1985a) *Biochemistry 24*, 7342–7348.

Kinet, J.-P., Quarto, R., Perez-Montfort, R., & Metzger, H. (1985b) *Biochemistry 24*, 7342–7348.

Kinet, J.-P., Metzger, H., Hakimi, J., & Kochan, J. (1987) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 46*, 1346 (Abstr. 6006).

Kochan, J., Perkins, M., & Ravetch, J. V. (1986) *Cell (Cambridge, Mass.) 44*, 689–696.

Kulczycki, A., Jr., McNearney, T. A., & Parker, C. W. (1976) *J. Immunol. 117*, 661–665.
Kumar, N., & Metzger, H. (1982) *Mol. Immunol. 19*, 1561–1567.
Lathe, R. (1985) *J. Mol. Biol. 183*, 1–12.
Lehrach, H., Diamond, D., Wozney, J. M., & Boedker, H. (1977) *Biochemistry 16*, 4743–4751.
Lewis, V. A., Koch, T., Plutner, H., & Mellman, I. (1986) *Nature (London) 324*, 372–375.
Liu, F.-T., & Orida, N. (1984) *J. Biol. Chem. 259*, 10649–10652.
Liu, F.-T., Albrandt, K., Mendel, E., Kulczycki, A., Jr., & Orida, N. K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4100–4104.
Ludin, E., Hofsteller, H., Sarfati, M., Levy, C. A., Suter, V., Alaimo, D., Kilcherr, E., Frost, H., & Delespesse, G. (1987) *EMBO J. 6*, 109–114.
Kilcherr, E., Frost, H., & Delespesse, G. (1987) *EMBO J. 6*, 109–114.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning, a Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Martens, C. L., Huff, T. F., Jardieu, P., Trounstine, M. L., Coffman, R. L., Ishizaka, K., & Morr, K. W. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2460–2464.
Maxam, A. M., & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 560–564.
McPhaul, M., & Berg, P. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8863–8867.
Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K., & Green, M. R. (1986) *Nucleic Acids Res. 12*, 7035–7056.
Metzger, H., Alcaraz, G., Hohman, R., Kinet, J.-P., Pribluda, V., & Quarto, R. (1986) *Annu. Rev. Immunol. 4*, 419–470.
Perez-Montfort, R., Kinet, J.-P., & Metzger, H. (1983a) *Biochemistry 22*, 5722–5728.
Perez-Montfort, R., Fewtrell, C., & Metzger, H. (1983b) *Biochemistry 22*, 5733–5737.
Portnoy, D. A., Erickson, A. H., Kochan, J., Ravetch, J. V., & Unkeless, J. C. (1986) *J. Biol. Chem. 261*, 14697–14703.
Pure, E., Luster, A. D., & Unkeless, J. C. (1984) *J. Exp. Med. 160*, 606–611.
Quarto, R., & Metzger, H. (1986) *Mol. Immunol. 23*, 1215–1223.
Ravetch, J. V., Luster, A. D., Weinshank, R., Kochan, J., Pavlovec, A., Portnoy, D. A., Hulmes, J., Pan, Y.-C. E., & Unkeless, J. C. (1986) *Science (Washington, D.C.) 234*, 718–725.
Rivnay, B., & Metzger, H. (1982) *J. Biol. Chem. 257*, 12800–12808.
Rivnay, B., Wank, S. A., Poy, G., & Metzger, H. (1982) *Biochemistry 21*, 6922–6927.
von Heijne, G. (1983) *Eur. J. Biochem. 133*, 17–21.
von Heijne, G. (1986) *Nucleic Acids Res. 14*, 4683–4690.