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Human and rat mast cell high-affinity immunoglobulin E receptors: Characterization of putative α-chain gene products

(cDNA cloning/allergy)

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ABSTRACT **We have cloned and determined the entire nucleotide sequence of cDNAs corresponding to the putative α subunits of the human and rat mast cell high-affinity IgE receptors. Both human and rat cDNAs encode an NH_2-terminal signal peptide, two immunoglobulin-like extracellular domains (encoded by discrete exons), a hydrophobic transmembrane region, and a positively charged cytoplasmic tail. The human and rat α subunits share an overall homology with one another and the immunoglobulin gene family, suggesting that they arose from a common ancestral gene and continue to share structural homology with their ligands. In addition, the rat gene is transcribed into at least three distinct forms, each of which yields a somewhat different coding sequence.**

The IgE receptor plays a central role in allergic disease, coupling allergen and mast cell to initiate the inflammatory and immediate hypersensitivity responses that are characteristic of disorders such as hay fever and asthma (1). Considerable evidence indicates that this response occurs when two or more high-affinity IgE receptors are crosslinked via IgE molecules that in turn are bound to an allergen (antigen) molecule (1–5). This perturbation brings about the release of histamine and proteases from the cytoplasmic mast cell granules and the synthesis of prostaglandins and leukotrienes—potent, short-range effectors that mediate the hypersensitivity response.

The complete amino acid sequence of the IgE portion of this ligand–receptor couple is known (6–8), and considerable progress has been made in characterizing the rat IgE receptor at the protein level (1, 4). It consists of three subunits: α, a heavily glycosylated subunit of 50–60 kDa exposed to the outer surface of the cell and bearing the IgE-binding site; and β and γ, two nonglycosylated intramembrane components of approximately 33 and 20 kDa, respectively. Several monoclonal antibodies that competitively inhibit the binding of IgE and immunoprecipitate the receptor complex have been raised against the rat IgE receptor, allowing further characterization of the receptor subunits (9).

Recently, by using methods similar to those described here, a cDNA corresponding to the putative α subunit of the rat high-affinity IgE receptor has been sequenced by Kinet *et al.* (10). Our parallel studies have resulted in cloning and sequencing cDNAs corresponding to these polypeptides from both man and rat.¶ From these, we deduced the entire amino acid sequences of both proteins. A number of interesting features can be discerned by comparing the human and rat sequences to one another and to other receptors and immunoglobulin-like molecules.

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MATERIALS AND METHODS

IgE Receptor Protein Purification, Tryptic Peptide Preparation, and Sequence Determination. Rat basophilic leukemia (RBL-2H3) cells were solubilized and incubated overnight at 4°C with monoclonal anti-rat mast cell IgE receptor antibody (BC4) coupled to Sepharose 4B beads (8). The beads were washed, and the bound proteins were eluted with 5% acetic acid and lyophilized. Aliquots were analyzed by NaDodSO$_4$/PAGE followed by silver staining. The sample was reduced with 2 mM dithiothreitol in 6 M guanidine hydrochloride/100 mM Tris, pH 8.3/1.0 mM EDTA at 37°C under N_2 and was S-carboxymethylated with 10 mM iodoacetic acid. The samples were desalted by HPLC and treated with TPCK (L-1-tosylamido-2-phenylethyl chloromethyl ketone)-treated trypsin in 100 mM Tris (pH 7.2). The resulting tryptic peptides were separated by HPLC on a Vydac C_4 column. Peptides were sequenced by using an Applied Biosystems (Foster City, CA) vapor-phase amino acid sequencer. NaDodSO$_4$/PAGE was performed as described by Laemmli (11). Silver staining of the gels was as described (12). The immunoblots and protein iodination studies were carried out as described (9).

Recombinant DNA/RNA Methodology. RNAs were extracted by the guanidinium isothiocyanate method (13). Rat liver DNA was extracted as described (13). Oligonucleotides used for probes and sequencing primers were synthesized by an automated DNA synthesizer (models 380A and B, Applied Biosystems). Nucleotide sequences were determined by the dideoxy chain-termination method (14) using alkali-denatured plasmid DNA. Poly(A)$^+$ RNAs were prepared by using oligo(dT)-cellulose columns (15). cDNA libraries were constructed as described (16, 17). Colonies were screened as described (18).

Five micrograms of digested rat liver DNA was run on a 0.7% agarose gel, and 10 μg of total RNA was run on a 1.0% agarose gel containing 6.6% formaldehyde, and both were blotted to nitrocellulose filters (13, 19). Filters were hybridized with nick-translated probes as described (13) and washed in 0.1× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) at 65°C (DNA blots) or 55°C (RNA blots). RNA was labeled using T7 RNA polymerase transcribing a *Nhe* I-digested subclone in pGEM3 and was used for probes for RNase protection assays (20).

Computer Analysis. Computer-assisted analysis was done with software from the Genetics Computer Group of the University of Wisconsin (versions 4 and 5) (21).

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¶These sequences are being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg), [accession nos. J03605 (human sequence) and J03606 (rat sequence)].

RESULTS AND DISCUSSION

Purification of Rat Mast Cell IgE Receptor and Amino Acid Sequence of Tryptic Peptides of α Chain. The IgE receptor was purified from solubilized RBL-2H3 cell extracts by using an anti-IgE receptor antibody column and was visualized by silver staining of the $NaDodSO_4$/PAGE gels (Fig. 1 *A* and *B*). As expected, there were bands corresponding to α (a broad band between 45 and 66 kDa with two sharper bands seen immediately below), β (a doublet of bands seen at approximately 30 kDa), and γ (material at about 20 kDa) chains of the receptor. IgE was shown to bind to the α chain (Fig. 1*C*). The different receptor components were further purified by elution from $NaDodSO_4$/PAGE (Fig. 1*B*). Because of the broadness of the α-chain band, it was divided into two parts, a leading and a trailing edge, both of which were essentially identical (data not shown).

Since the N-terminal eluted samples seemed to be blocked, we purified tryptic peptides by HPLC and then determined the amino acid sequence of several of these peaks (Fig. 1*D*) (approximately 100 pmol). Among these peptide sequences, peptide 4 (peak 4) showed significant homology to a sequence near the NH_2 terminus of the mouse receptor for the Fc fragment of IgG (Fc_γ) (22), suggesting an analogous sequence in the IgE receptor subunit. Therefore, an oligonucleotide probe corresponding to this peptide was prepared to detect near-full-length cDNA clones for the IgE receptor.

Isolation of Rat Mast Cell IgE Receptor α-Chain cDNA Clones and Determination of Its Sequence. From the sequence of peptide 4, the least codon-redundant portion, Asp-Pro-Pro-Trp-Ile, was chosen to make 32 possible variations of the 14-mer mixture of the oligonucleotide, 5′ ATCCANGGNGRTC 3′, in which N is G, A, T, or C, and R is A or G. About 7×10^4 independent cDNA clones of RBL-2H3 cell mRNA were screened, and three positive clones were identified. The nucleotide sequence of two of the three clones that showed similar restriction patterns was determined by the strategy shown in Fig. 2*A Left*. These clones have exactly the same sequence except for one deletion [21 base pairs (bp)] in pAS-r-IgER-5A (clone 5A) and two deletions (163 and 8 bp) in pAS-r-IgER-2A (clone 2A) (Fig. 2 *A Left* and *B*). Since we know that the majority of IgE receptor mRNA has no deletions (see below), we deduced the primary structure of rat IgE receptor α chain from the 735-bp open reading frame of undeleted sequence (Fig. 2*B*). It encodes a 245-amino acid peptide containing perfect matches to the peptide sequences determined by amino acid sequencing (Fig. 2*B*). This amino acid sequence differs from that deduced by Kinet *et al.* (10) as a result of single-base differences at codon positions 2 and 237 and a single-base frameshift deletion in codon 245. Thus, our peptide differs at positions 2, 237, and 245 and is 5 amino acids shorter than that of Kinet *et al.* (10). Our entire sequence has been confirmed by sequencing the corresponding rat genomic clones (I.T. and A.S., unpublished data).

The hydrophobicity profile (not shown) suggests that the IgE receptor α chain has an extracellular NH_2-terminal portion (181 amino acid residues) that crosses the cell membrane only once (19 amino acids), and that it has a short cytoplasmic tail (22 amino acids). We tentatively assign the NH_2 terminus as an alanine residue at position 24 from the consensus sequence around the cleavage sites of the leader peptides (23) (Fig. 2*B*).

An unusual feature of the putative transmembrane sequence is a negatively charged aspartic acid residue at position 218. This might explain the need for ancillary chains for the α subunit to be expressed on the mast cell surface (see below). The T-cell antigen receptor α and β subunits, for example, have a positively charged residue in the transmembrane region and require T3 antigen for surface expression (24–26). Characteristically, the cytoplasmic portion of the IgE receptor has many positively charged residues (seven lysines) and is very hydrophilic. In addition, there are seven possible N-linked glycosylation sites and many serine and threonine residues that may be targets for O-linked glycosylation in the extracytoplasmic region.

Isolation of the Human Mast Cell IgE Receptor α-Chain cDNA Clone and Its Comparison to the Rat Sequence. To clone the human α-chain cDNA, a cDNA library was prepared from a human mast cell line known to produce IgE receptors (KU812). About 9×10^4 colonies were screened

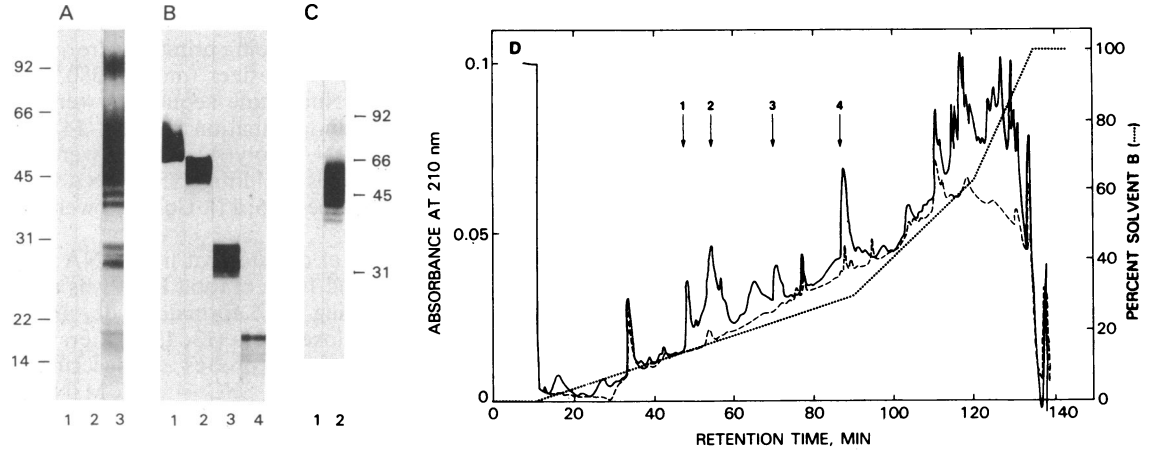

FIG. 1. Purification of the rat mast cell IgE receptor and tryptic peptides of the α chain. (*A*) $NaDodSO_4$/PAGE analysis of the purified receptor complex. Silver-stained material was eluted from Sepharose 4B alone (lane 1), from normal mouse immunoglobulin-coupled beads (lane 2), and from monoclonal antibody BC4-coupled beads (lane 3). Material obtained from about 1.5×10^6 solubilized RBL-2H3 cells was applied to the gel. (*B*) $NaDodSO_4$/PAGE analysis of purified receptor components. Loaded samples for each lane are as follows: 1 and 2, α-chain leading and trailing edges; 3, β chain; 4, γ chain. (*C*) Immunoblotting of the purified receptor. Purified receptor complex (as shown in lane 3 of *A*) was transferred to a nitrocellulose filter and was bound to ^{125}I-labeled IgG (lane 1) or ^{125}I-labeled IgE (lane 2). (*D*) Purification of tryptic peptides of α chain. Tryptic peptides from the α-chain trailing edge were separated by HPLC on a Vydac C_4 column. Elution was with gradients of acetonitrile (final percentage, 90%) in 0.1% trifluoroacetic acid. The solid line indicates the absorbance of the sample, and the broken line indicates that of the solvent baseline. Dots show the concentration of the solvent. Peaks indicated by arrows were subjected to amino acid sequencing. Peptide sequences obtained from these peaks are: Trp-Ile-His-Asn-Asp-Ser-Ile-Ser-Asn-Xaa-Lys (peak 1), Tyr-Ser-Tyr-Asp-Ser-Asn-Xaa-Ile-Ser-Ile-Arg and Ile-Leu-Thr-Gly-Asp-Lys-Val-Thr-Leu-Ile-Xaa-Asn-Gly (peak 2), Val-Ile-Tyr-Tyr-Lys (peak 3), and Ser-Val-Val-Ser-Leu-Asp-Pro-Pro-Trp-Ile-Arg (peak 4).

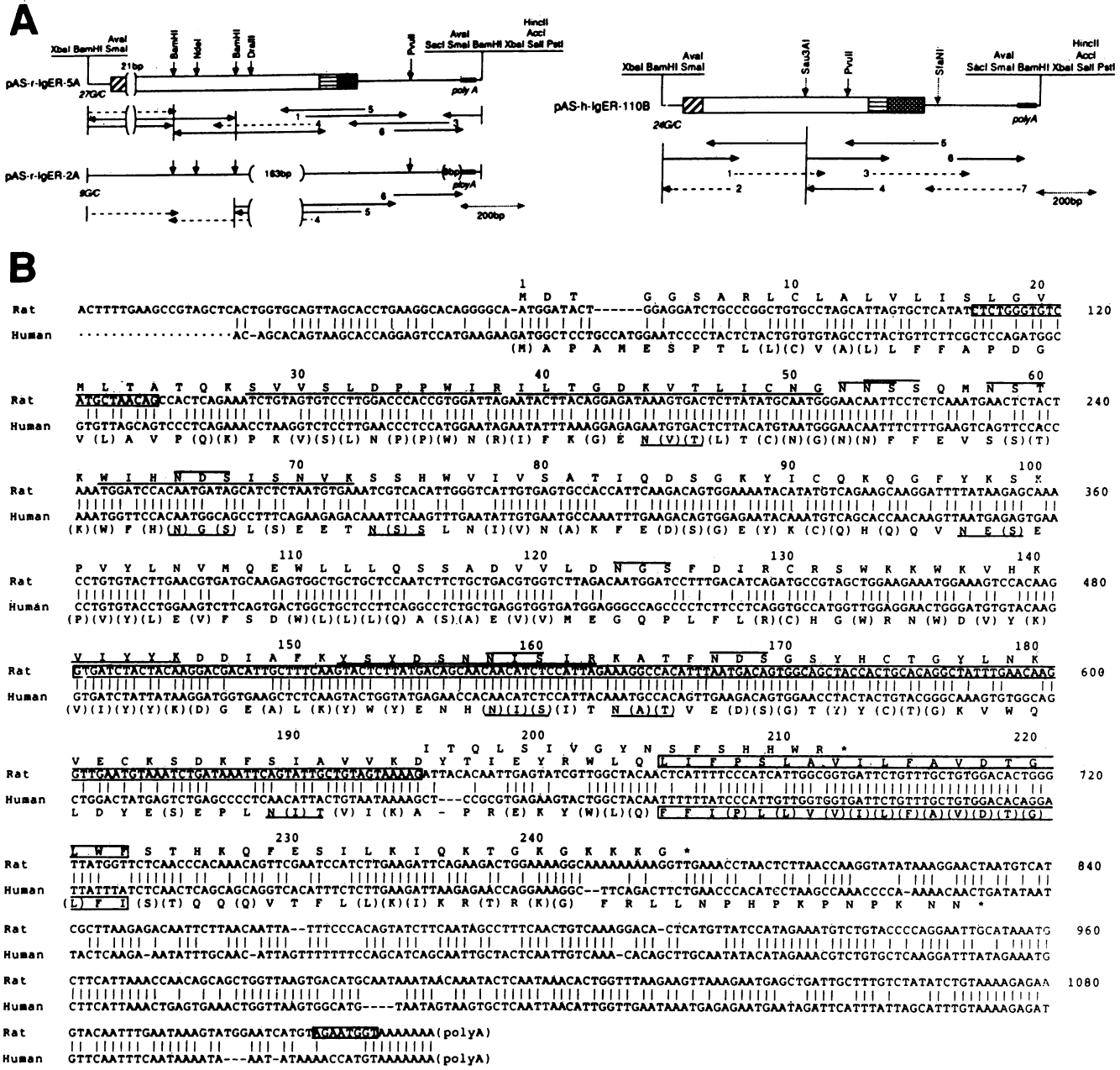

FIG. 2. Nucleotide sequence analysis of rat and human mast cell IgE receptor α-chain cDNA clones. (*A*) Restriction maps and sequencing strategies. (*A Left*) Rat clone. These cDNA clones were isolated by hybridization to a probe composed of a mixture of 32 ^{32}P-labeled 14-mer oligonucleotides. Hybridization was performed in 1 M NaCl with 3.2×10^7 cpm of probe per ml at 42°C for 15 hr, and washing was done twice in 4× SSC containing 0.1% $NaDodSO_4$ at 42°C for 15 min. Restriction enzyme sites in the inserts are shown by vertical arrows and in the polylinker are shown on horizontal lines. Boxes on the map of pAS-r-IgER-5A indicate the protein coding regions: hatched boxes, for leader peptide; open boxes, for extracytoplasmic regions; boxes filled by horizontal lines, for transmembrane regions; and cross-hatched boxes, for cytoplasmic regions. Horizontal arrows indicate the DNA sequences determined. Arrows with numbers indicate those sequences determined by using specific oligonucleotides synthesized from previously determined sequences (15–25 mer). Broken arrows indicate that these sequences were determined by using the original clones to overpass subcloning sites. (*A Right*) Human clone. This cDNA clone was isolated by hybridization with nick-translated *Hpa* II (position 46)–*Pvu* II (position 970) fragment of rat cDNA clone 2A/5A. Hybridization was done in 6× SSC containing 50% formamide and 10% dextran sulfate at 42°C for 15 hr; the filters were then washed twice in 0.1× SSC containing 0.1% $NaDodSO_4$ at 55°C for 15 min. The restriction enzyme map, location of peptide coding regions, and sequencing strategy are indicated with the same conventions as in *A Left*. (*B*) Nucleotide sequences, deduced protein sequences, and their comparison. Rat mast cell IgE receptor α-chain nucleotide sequence and its translated protein sequence (above the nucleotide sequence) in the one-letter code are shown. The sequence resulting from the 163-bp deletion is shown above the full-length sequence. The amino acid sequences that matched with the determined tryptic peptides are indicated by underlining. Deleted nucleotides in clone 5A (21 bp) and clone 2A (163 and 8 bp) are boxed. Corresponding human sequences are shown under them. Gaps are introduced to maximize the homology indicated by vertical lines. Matched nucleotides are connected, and the amino acids of the human sequence conserved with the rat sequence are shown in parentheses. Possible N-linked glycosylation sites are indicated by overlining (rat) and underlining (human). Putative transmembrane regions are boxed.

with the rat α-chain cDNA probe at 55°C in 1× SSC; three positive colonies were found. Among these, one, which had the largest insert (pAS-h-IgER-110B), was further characterized. Both nucleotide and deduced amino acid sequences were compared with the rat sequence (Fig. 2*B*).

Overall homologies between human and rat sequences are

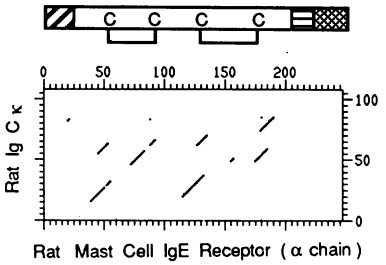

FIG. 3. Homologies between rat mast cell IgE receptor α chain and immunoglobulin κ-chain constant region (C_κ) peptides. Dot-matrix analysis was done with the "compare and dotplot" program of University of Wisconsin Genetics Computer Group (version 4); the program counts only identical amino acids. Comparison was done at window 30 and stringency 7.0. Schematic representation of the rat IgE receptor is also shown above the plot with the same conventions as in Fig. 2*A*. Rough positions of four cysteine residues that may make disulfide bonds are also shown in this scheme as capital letters.

68% and 45% at the nucleotide and protein level, respectively. There are several stretches of conserved amino acids between the extracytoplasmic portions of the human and rat polypeptides. Most of these are also conserved—although to a lesser extent—in the mouse Fc_γ (IgG) receptors (see below), suggesting that they may be important for the common structure of receptors to the Fc portion of immunoglobulin. The conserved stretches between amino acid positions 140 and 146 are the only regions that are not conserved in Fc_γ (IgG) receptors. Therefore, this region may have some role specific to IgE receptors, such as in the binding of IgE or in the receptor's interaction with other specific component(s). The transmembrane region is also well conserved between the rat and human IgE receptor, suggesting that it is required for interaction with other component(s) and signal transduction.

The carboxyl termini of the rat and human receptors are surprisingly different. The human subunit has an extra 12 amino acids, which may have arisen by a frameshift mutation. The nucleotide sequence of this region has been confirmed by sequencing another independent human cDNA clone to rule out a cDNA cloning artifact.

Homology of the Mast Cell IgE Receptor α Chain to Immunoglobulin-like Proteins. Kinet *et al.* (10) have pointed out that the Fc_γ (IgG) receptor α (22) has homology throughout its extracytoplasmic and transmembrane regions with the rat mast cell IgE receptor α chain. A data-base comparison of the mast cell IgE receptor to other sequenced proteins revealed significant homology to immunoglobulins. One example, a dot-matrix comparison between the rat κ-chain constant region and the rat IgE receptor, is shown in Fig. 3. There are two internal repeats homologous to the immunoglobulin domain in the extracytoplasmic region of the mast cell IgE receptor. These correspond to discrete exons that we have identified in the rat genomic sequence (I.T. and A.S., unpublished results). Each repeat unit conserves two cysteine residues, which may be important in forming domains by disulfide bonding. Amino acids conserved among immunoglobulins are generally conserved within these two repeats; for example, the Asp-Xaa-Gly-Xaa-Tyr-Xaa-Cys sequence in front of the second cysteine in each repeat unit corresponds to one of the highly conserved sequences in the immunoglobulin variable region genes (27). This homology indicates that the mast cell IgE receptor is a member of the immunoglobulin supergene family and provides an example in which ligand and receptor arise from a common ancestral gene (22, 28). In contrast to the above-noted similarities, there is no significant homology between

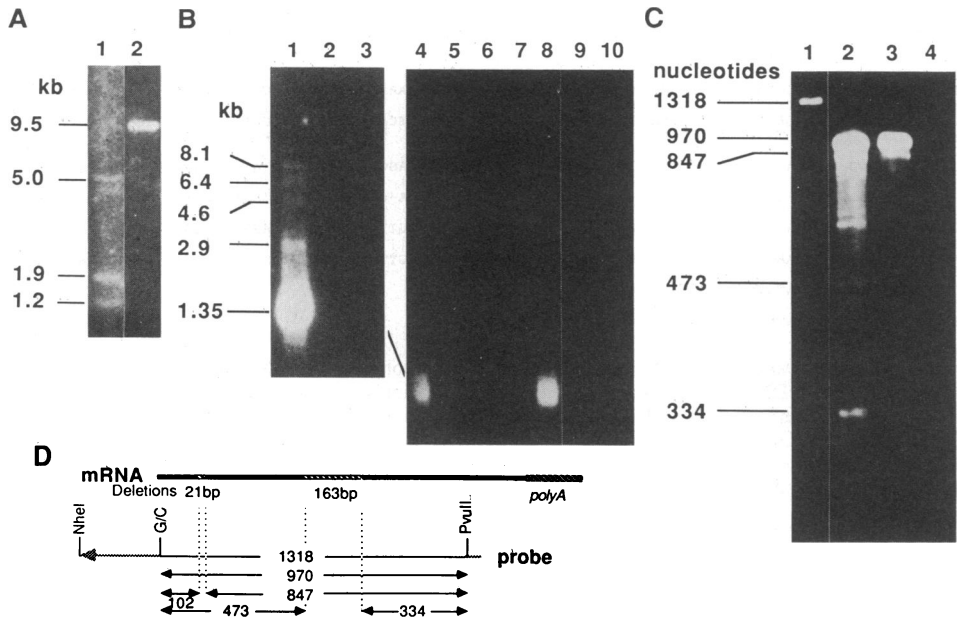

FIG. 4. Genomic representation and tissue-specific expression of the rat mast cell IgE receptor α-chain gene. (*A*) Southern blot analysis. *Hin*dIII (lane 1) and *Eco*RI (lane 2) digests of rat liver DNA were hybridized to the complete insert of clone 2A/5A. Sizes of hybridized fragments are shown. (*B*) RNA blot-hybridization analysis. Blots of various RNAs were hybridized to the *Hpa* II (position 46)–*Pvu* II (position 970) fragment of clone 2A/5A. Origins of RNAs in lanes are as follows: 1, RBL-2H3; 2, rat heart ventricular muscle; 3, rat cerebellum; 4, ASFTL [Abelson virus-transformed mouse mast cell line (34)]; 5, BW5147 (mouse T-lymphocyte cell line); 6–8, NFS60, DA1 and DA3 (mouse myeloid-monocyte precursor leukemic cell lines) (35); 9 and 10, RNK8 and RNK16 (rat natural killer-like cell lines) (36). Sizes of hybridized fragments are indicated. (*C*) RNase protection analysis. Twenty-five and 10 μg of RBL-2H3 total RNA (lanes 2 and 3) and 25 μg of yeast tRNA (lane 4) were hybridized to the probe in *D*. Undigested probes were run on lane 1. Sizes of the probes and protected fragments agreed with prediction (see *D*) as is indicated. (*D*) Probes for RNase protection and predicted sizes of protected fragments. The probe used for the RNase protection assay (*C*) is shown in the top line with its size. The predicted protected fragments from the full-length form and from two deleted forms of mRNA are indicated by arrows under the probe with their sizes.

the mast cell IgE receptor and the lymphocyte IgE receptor (29–31) or the two cloned IgE binding factors (32, 33).

Genomic Representation and Specificity of Rat Mast Cell IgE Receptor α-Chain mRNA Expression. Only a few bands in rat liver DNA hybridize to the IgE receptor cDNA probe (Fig. 4*A*), indicating that the mast cell IgE receptor α chain is a unique-copy gene. We have also determined the tissue specificity of IgE receptor mRNA expression in various tissues and cell lines of rat and mouse (Fig. 4*B*). RBL-2H3 cells yield an intense 1.35-kilobase (kb) band and several higher molecular mass bands. The higher molecular mass bands are likely to be unspliced precursors, with the largest band (8.1 kb) roughly corresponding to the size of the gene. Among the tissues and cell lines studied, only mast cells and an interleukin 3-dependent myeloid-monocyte precursor express this gene, indicating that its expression is highly specific.

To account for the deletions noted in the rat cDNA clones, we have carried out RNase protection experiments to determine the major mRNA species in RBL-2H3 cells. A full-length cDNA clone to be used as a probe was made by linking undeleted segments of the original cDNA clones (clone 2A/5A). The major protected species is 970 bases long and corresponds to the undeleted form of the mRNA (Fig. 4*C*, lanes 2 and 3). There are three minor bands corresponding to two deleted forms (847, 473, and 334 bases), corresponding to about 10% of the level of full-length mRNA. The expected 102-bp fragment was visible on longer exposures (not shown). These results indicate that the major species of mRNA is the undeleted form and that there are at least two (possibly three) minor deleted forms of α-chain mRNA. The sequence of the corresponding rat gene (I.T. and A.S., unpublished results) indicates that these mRNAs arise by alternative splicing of the genomic transcript.

To express functional IgE receptor, we have transfected simian virus 40 promoter-driven rat cDNA α-chain clones into a mouse mast cell-like line that had lost endogenous IgE receptor expression. Although the DNA was successfully transfected, neither IgE nor monoclonal antibody BC4 binding was detected (data not shown). This result may indicate that α chain alone cannot be expressed on the cell surface and that another chain(s) (β and/or γ) of the receptor complex is necessary, just as the T-cell antigen receptor requires T3 expression (26). Our preliminary experiments, which indicate that reconstitution of IgE binding activity in *Xenopus laevis* oocytes requires injection of a mixture of two size fractions of RBL-2H3 mRNAs, strongly support this possibility (P.N.B. and P.L., unpublished observations).

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