THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 267, No. 18, Issue of June 25, pp. 12782-12787, 1992
Printed in U.S.A.

The Gene and cDNA for the Human High Affinity Immunoglobulin E Receptor β Chain and Expression of the Complete Human Receptor*

(Received for publication, February 11, 1992)

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The high affinity IgE receptor (FcεRI) is a tetrameric hetero-oligomer composed of an α chain, a β chain, and two disulfide-linked γ chains. The β chain contains four transmembrane (TM) segments and long cytoplasmic domains that are thought to play an important role in intracellular signaling. We now report the structural characterization and the sequence of the complete human β gene and cDNA. The gene spans ~10 kilobases and contains seven exons. There is a single transcription initiation site preceded by a TATA box. The first exon codes for the 5′-untranslated region and a portion of the N-terminal cytoplasmic tail. TM-1 is encoded in exons 2 and 3, TM-2 in exons 3 and 4, TM-3 in exon 5, and TM-4 in exon 6. The seventh and final exon encodes the end of the C-terminal cytoplasmic tail and the 3′-untranslated sequence. The human β gene appears to be a single copy gene. Two corresponding transcripts, detected as a doublet around 3.9 kilobases, are present in cells of mast cell and basophil lineage from different individuals, but not in the other hematopoietic cells tested here. The human β protein is homologous to rodent β. The consensus amino acid sequences of human, mouse, and rat β show 69% identical residues.

Analysis of the surface expression of transfected receptors indicates that human αγ and αβγ complexes are expressed with comparable efficiency. Human β interacts with human α more efficiently than does rat β, and both rat and mouse β interact with their corresponding α more efficiently than does human β, demonstrating a species specificity of the α/β interaction.

The high affinity IgE receptor (FcεRI) is responsible for initiating the allergic response (1). Binding of allergen to receptor-bound IgE leads to cell activation and the release of mediators (such as histamine) responsible for the manifestations of allergy. This receptor is a tetrameric complex ($\alpha\beta\gamma_2$) that is found on the surface of mast cells and basophils. The α and β subunits have not been detected in other hematopoietic cells (1, 2), although the γ chains of FcεRI are found in macrophages (3), NK cells (4), and T cells (5), where they associate with the low affinity receptor for IgG (FcγRIII) or with the T cell antigen receptor.

Complementary DNAs have been isolated for the α (6–10), β (11), and γ (9, 12, 13) chains in mouse, rat, and humans. The genes for α and γ both have been localized on human (14) and mouse (15, 16) chromosome 1. The gene for mouse β has been localized on mouse chromosome 19 by genetic linkage and is thought to be a single gene (16). The structures of the α gene (rat) (17) and of the γ gene (human) (13), but not of the β gene, have been characterized.

The molecular cloning of the subunits has permitted the reconstitution of surface-expressed receptor complexes by transfection. One of the surprising findings from these studies was the differential requirement for surface expression among the different species. Cotransfection of the three chains (α, β, and γ) is required to promote efficient surface expression of the rat (12) or mouse (9) receptor. By contrast, surface expression of the human αγ complex can be achieved by cotransfecting α and γ alone, suggesting that β is not necessary (18). This result (and our inability to clone human β) raised the interesting possibility that αγ complexes may exist naturally in human cells. To address this question and to study the possible regulation of human β expression, we have isolated and characterized the human β gene and cDNA.

EXPERIMENTAL PROCEDURES

Screening of cDNA and Genomic Libraries—The human basophil cDNA library and the human leukocyte genomic library have been described before (13). The human lung cDNA library (19) and a human skin cDNA library were kindly provided by Lawrence B. Schwartz (Medical College of Virginia, Richmond, VA).

The following probes were prepared for screening the various libraries: the *Eco*RI-*Eco*RV fragment of rat β (11) and the *Eco*RI fragment of mouse β (9), both of which contain the entire coding sequence of β and part of the 3′-untranslated region. Fragments of the coding region of rat β cDNA (bp[1] 1–304) and of mouse β cDNA (bp 433–708) were made by polymerase chain reaction (PCR). Multiple oligonucleotides corresponding to various regions of rat, mouse, and human β were synthesized on a Model 380A automated DNA synthesizer (Applied Biosystems, Inc., Foster City, CA). All double-stranded DNA probes were radiolabeled by random primer labeling, and the oligonucleotides by end labeling as described elsewhere (20).

Hybridization and washing conditions and procedures for plaque purification, subcloning, sequencing, and DNA analysis were as described previously (13).

cDNA Synthesis Using PCR—Basophils from 240 ml of blood were purified by double Percoll gradients as previously described (21), and basophil RNA was extracted by the guanidinium isothiocyanate method (20). Two μg of total RNA were reverse-transcribed with Superscript reverse transcriptase (Bethesda Research Laboratories) using a random 9-mer primer as recommended by the manufacturer. One-twentieth of the reaction product was amplified using the following primers: a 23-mer complementary to nucleotides −2 to +21 of the human β coding sequence and, as backward primer, a degenerated 21-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M89796.

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[1] The abbreviations used are: bp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction; TM, transmembrane.

mer of the mouse and rat β sequences starting 32 nucleotides after the stop codon. Temperature cycles were as follows: 1 cycle of 2 min at 95 °C, 2 min at 94 °C, 5 min at 37 °C, and 40 min at 72 °C; 4 cycles of 40 s at 94 °C, 1 min at 37 °C, 4 min at 72 °C; and 36 cycles of 40 s at 94 °C, 1 min at 50 °C, and 4 min at 72 °C, followed by a single 15-min extension. One μl of this reaction was reamplified, omitting cycles 2–5, and the amplification product was subcloned into pCR1000 using the TA cloning kit (Invitrogen, San Diego, CA).

Direct Sequencing of Gene Fragments Obtained by PCR—Purified insert-containing phage DNA from the leukocyte genomic library was linearized with *Not*I, and 100 ng were amplified with primers flanking the region to be sequenced. DNA amplification was achieved using 40 of the following cycles: denaturation for 1 min at 94 °C, annealing for 2 min at 45–50 °C, and extension for 3–6 min at 72 °C. Subsequently, 1 μl of the amplified material was reamplified in three separate reactions (50 μl) under identical conditions, omitting one of the two primers to generate single-stranded DNA. The three reactions were pooled, applied to an Ultrafree MC 30.000 spin column (Millipore, Bedford, MA), and washed four times before being evaporated by vacuum. The single-stranded DNA was sequenced using the omitted primer or an internal primer. Comparison of sequences obtained by this method or by sequencing nonamplified fragments being subcloned in pGEM vectors revealed no differences.

Sequencing Transcription Start Site—PCR was used to define the transcription start site. Procedures published elsewhere (22) were modified as follows. Five μg of RNA were reverse-transcribed as detailed above using a primer corresponding to nucleotides +451 to 429 of the coding region. The resulting product was washed on a Centricon 100 column (Amicon Corp., Beverly, MA), and poly(A) tails were added at both ends using terminal transferase (Bethesda Research Laboratories) as recommended by the manufacturer. One-sixth of this reaction was amplified with the following two primers: a 33-mer consisting of the M13 primer sequence following by 17 T residues and, for the 3′ end, a primer derived from nucleotides 331 to 308 of the human β coding region sequence. Subsequently, an internal amplification was performed exchanging the 3′ primer for one equivalent to nucleotides +189 to 169. Finally, single-stranded DNA was produced for sequencing using an oligonucleotide corresponding to nucleotides 54 to 33 as the only primer. For all PCRs, the annealing temperature was 45 °C, and the extension time was 3 min.

Analysis of Transcription Start Site by 5′ Extension—An end-labeled oligonucleotide corresponding to the negative strand at nucleotides 54 to 33 after the start codon was hybridized overnight at 42 °C with either 10 μg of total RNA from enriched basophils (see above) or 10 μg of tRNA, followed by extension with Superscript reverse transcriptase at 45 °C for 90 min. The primer-extended products were separated on urea 5% polyacrylamide gel in parallel with the sequencing reactions of the genomic DNA.

Other Methods—Northern and genomic Southern blots were performed as described elsewhere (20). The various cDNAs were subcloned into the eukaryotic expression vector pCDL-SR(α) for the transfection studies (23). COS-7 cells were transfected by the standard DEAE-dextran method (24), except that a 3-min incubation of the transfected cells in 10% dimethyl sulfoxide in media was added after the chloroquine treatment.

RESULTS AND DISCUSSION

Isolation, Mapping, and Sequencing of Human FcεRI β Gene—We first attempted to isolate human β cDNA clones by screening a human mast cell cDNA library with full-length rat and mouse cDNA probes. These probes were radiolabeled and used to screen 7 × 10^5 colonies. Four clones were isolated, all of which contained a 153-bp insert with 73% homology to rat β cDNA. The sequence of this insert corresponded to a portion of β that includes the intracellular loop and the third transmembrane domain. These four identical clones are the likely result of library amplification of a single clone generated by recombinations. We screened two additional libraries: another mast cell cDNA library and a cDNA library derived from basophil-enriched leukocytes. The latter library was used earlier to isolate human γ cDNA clones. A total of 10^7 independent cDNA clones were screened with a panel of murine probes and oligonucleotides and with the 153-bp human β probe. However, no additional clones were isolated.

We then screened 6 × 10^5 independent genomic clones from a human genomic leukocyte library with the radiolabeled 153-bp human probe, and 10 clones with average size inserts of 25 kb were isolated. These clones all hybridized with two 20-mer oligonucleotide probes corresponding to the beginning and the end of the rat β coding sequence. Four different restriction patterns could be generated from the 10 clones. However, Southern blots with various oligonucleotide probes scanning different regions of the rat β coding sequence indicated that the four restricted patterns were not the product of different genes. Rather, the clones showed differences in the lengths of the sequences flanking the β gene (data not shown).

One clone containing a 25-kb insert was chosen for further characterization, mapping, and sequencing. A restriction map shown in Fig. 1 was constructed by complete and incomplete digestions with the restriction endonucleases *Hin*dIII, *Pst*I, *Bam*HI, *Xba*I, *Sma*I, and *Kpn*I. A 3.2-kb *Hin*dIII fragment was found to hybridize with oligonucleotide probes corresponding to the start codon and transmembrane regions I and II of rat β. A 2.8-kb *Sma*I fragment hybridized with rat β probes of transmembrane regions III and IV, and a 4.5-kb *Sma*I fragment with probes of the stop codon region. The three fragments were subcloned into pGEM3zf(+) or-(−) and sequenced in full (Fig. 2). The fragment corresponding to the 0.9-kb gap between the *Hin*dIII and 2.8-kb *Sma*I fragments was produced by PCR and sequenced. We also confirmed by PCR that the two *Sma*I fragments were adjacent to each other.

By comparing the sequences of the human β gene and of the rat β cDNA (Fig. 3), we localized seven homologous regions that may correspond to seven different exons.

Synthesis of Human β cDNA Coding Sequence—To confirm the sequence of the exons and to define the intron-exon borders, human β cDNA was synthesized by reverse transcription of RNA purified from basophil-enriched leukocytes, followed by amplification of the reverse transcripts using PCR (described under "Experimental Procedures"). This amplified product extended from two nucleotides preceding the start codon to 32 nucleotides following the stop codon. The cDNA sequence was found to be identical to the corresponding sequence of the human β gene. This confirmed that the coding sequence of human β is contained in seven exons. Furthermore, the comparison of cDNA and gene sequences and the detection of consensus sequences for intron-exon borders in the human β gene allow for a precise determination of these borders. The 5′ borders of the six intervening introns invariably start with GT, and the 3′ borders end with AG.

Analysis of Human β Transcripts—To evaluate the lengths of the 5′- and 3′-untranslated sequences, we analyzed the size of human β transcripts. RNAs from basophil-enriched leukocytes obtained from different individuals were hybridized by Northern blotting with the radiolabeled 153-bp human β

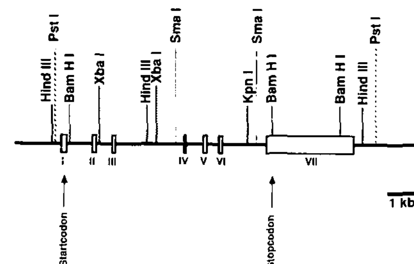

FIG. 1. **Structure of human β gene.** Restriction map and intron-exon structure are shown. The positions of the seven exons are depicted by *boxes*. The locations of the start and stop codons are indicated.

FIG. 2. **Nucleotide sequence of human FcεRI β chain gene.** The seven exons are shown in *boldface type*. The numbering of nucleotides is relative to the start codon. The TATAA box, translation initiation codon (ATG), and termination codon (TAA) and the potential polyadenylation signals (AATAAA) are *underlined*. Bases that were not determined with certainty are denoted as *N*.

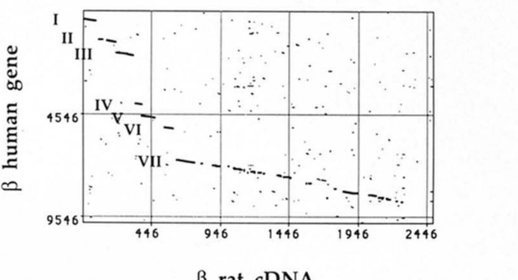

FIG. 3. **Comparison of human β gene and rat β cDNA sequences by dot matrix plot.** The Pustell DNA matrix of the Macvector program was used with a window of 30 nucleotides and a minimum score of 63%. The Roman numerals indicated on the left correspond to the seven exons.

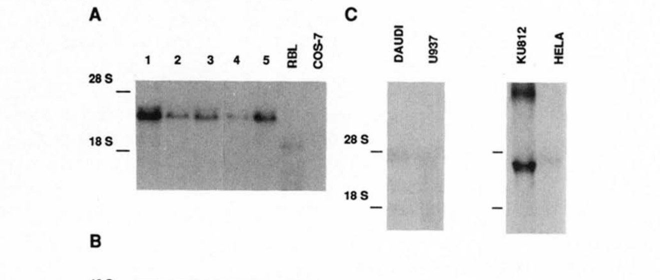

FIG. 4. **Presence of transcripts in basophils.** Ten μg of total RNA from basophil-enriched leukocytes and various other cells were fractionated on a denaturing agarose gel before being transferred to Nytran membranes and hybridized with human β cDNA probes (nucleotides +306 to +456 in *A* and nucleotides −2 to +790 in *C*). The membrane shown in *A* was stripped and rehybridized with a full-length human α cDNA probe (*B*).

probe (Fig. 4*A*). Two transcripts around 3.9 kb were found in human basophils, but not in COS-7 cells. The human transcripts are substantially longer than their rodent counterparts (2.7 and 1.75 kb) (9, 11) as detected in RBL cells by cross-hybridization. This longer size may explain our failure to isolate human β cDNAs from the three oligo(dT)-primed libraries. Similar results were obtained with a full-length cDNA probe of human β (data not shown). Hybridization of the same RNAs with a human α cDNA probe revealed transcripts for α of the expected size (1.1 kb) (Fig. 4*B*). RNAs from different cell lines were also hybridized with a full-length human β cDNA probe (Fig. 4*C*). The message for human β was only detected in the basophil line KU812, but not in U937, Daudi, and HeLa cells. An additional band was seen in KU812 cells that could correspond to unspliced transcripts.

With an open reading frame of 732 bp and assuming 200 bp for the poly(A) tail, human β transcripts should contain ~3 kb of untranslated sequences. Fig. 3 shows that most of the untranslated sequences are in the seventh exon. We also explored the possibility that additional exons of 3′- or 5′-untranslated sequences had not yet been identified.

Characterization of 5′ End and of Transcription Initiation Site—The transcription start site was determined by sequencing directly a PCR-amplified product of the reverse-transcribed RNA as described under "Experimental Procedures." RNA from basophil-enriched leukocytes was reverse-transcribed from a primer of the human β coding sequence. Poly(A) tails were added to the reverse transcripts by treatment with terminal transferase, and the resulting cDNAs were amplified by PCR. Single-stranded DNAs (positive strands, poly(dT)-tailed) were then produced by asymmetric PCR and directly sequenced. The cDNA sequence of the

negative strand corresponding to the 5′ end of the RNA is shown in Fig. 5*A* and is compared to the relevant sequence of the β gene. The perfect match between the two sequences ends after GGGTT. Then, the cDNA sequence reproducibly shows a C residue, which is not present in the gene, followed by the expected poly(A) tail. This additional C residue could correspond to the G residue of the cap structure and indicate the location of the start site.

Experiments of 5′ extension (Fig. 5*B*) confirmed that there is a major start site in this area (~11 nucleotides 3′ of the position described above), although it is difficult to exclude the possibility that the faint bands seen below and above the major start site could correspond to minor start sites. However, the presence of a TATAAA box found in the 5′ sequence supports the existence of a unique start site. In addition, the location of the TATAAA box (usually 25 nucleotides 5′ of the start site) is more consistent with the precise localization of the start site as shown in Fig. 5*A*. Indeed, the TATAAA box is located between nucleotides 29 and 24 upstream of this start site. Taken together, our data indicate that the human β mRNA starts with the sequence AACCC (see Figs. 2 and 5*A*) and has 102 bp of 5′-untranslated sequence.

Characterization of 3′ End—A comparison between the rat β cDNA and human β gene sequences (Fig. 3) shows that the seventh exon of the β gene extends from at least nucleotide 6773 to at least nucleotide 8910. But, an additional 3′-untranslated sequence (~800 bp) had to be found to fully account for the 3.9-kb transcripts. To analyze whether the missing sequence was part of the seventh exon or of other undetected exons, we prepared three probes from the β gene to test their reactivity with β transcripts. These transcripts hybridized on Northern blots with both the *Nsi*I-*Bam*HI fragment (nucleotides 8460–9250) and the *Bam*HI-*Sph*I fragment (nucleotides 9250–9714), but not with the fragment 3′ of the *Sph*I site (data not shown). Interestingly, two polyadenylation signals (AATAAA) were found at nucleotides 9663 and 9758 (Fig. 2). Therefore, this region is likely to correspond to the end of exon 7. We suggest that both polyadenylation signals could be used to create the apparent doublet of transcripts around 3.9 kb (see Fig. 4).

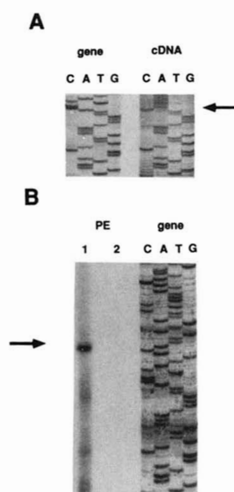

FIG. 5. **Determination of transcription initiation site.** *A*, RNA from basophils was reverse-transcribed, poly(A)$^+$-tailed at both ends with terminal transferase, and amplified by PCR. The amplified product (cDNA) and the genomic DNA (gene) were sequenced with an identical primer, and the respective sequencing reactions were run in parallel on a 8% acrylamide gel. The *arrow* marks the transcription start site. *B*, RNA from basophils (*lane 1*) or tRNA (*lane 2*) was used in the primer extension (*PE*), and the extended products were analyzed on a urea-5% polyacrylamide gel in parallel with the sequencing reactions of the genomic DNA. The *arrow* marks the transcription start site.

Organization of Human β Gene—Taken together, our data indicate that the human β gene contains seven exons and six introns and spans ~10 kb. Exon 1 codes for 102 bp of 5′-untranslated sequence and the first 18 amino acid residues of the N-terminal cytoplasmic tail. Exon 2 encodes the remaining of the cytoplasmic tail and the first 3 residues of TM-1. Exon 3 codes for the remainder of TM-1, the first extracellular loop, and the first half of TM-2. Exon 4 encodes the second half of TM-2 and a portion of the cytoplasmic loop. Exon 5 codes for the last 3 residues of the cytoplasmic loop, TM-3, and most of the second extracellular loop. Exon 6 codes for the last 2 residues of the extracellular loop, TM-4, and the first quarter of the C-terminal cytoplasmic tail. Finally, exon 7 codes for the remainder of the cytoplasmic tail and the long 3′-untranslated sequence.

Southern Blot Analysis—Digestion of genomic DNAs from five different individuals with *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Msp*I, and *Pvu*II and hybridization of these digests with a human cDNA probe (from start to stop codon) support the existence of a unique gene (Fig. 6). In addition, the lengths of the restriction fragments detected on the Southern blot are entirely consistent with the lengths predicted from the sequence of the gene. Three *Bam*HI sites (nucleotides 156, 6908, and 9250) are present in the gene. As expected, only one fragment (nucleotides 156–9250) is seen here because the other fragments should not hybridize with the cDNA probe. The two predicted *Bgl*II fragments (nucleotides +334 to +1766 and +1766 to +7419) and the two predicted *Hin*dIII fragments (nucleotides −454 to +2724 and +2724 to 100,042) were readily detected. The results obtained after *Eco*RI and *Pvu*II digestions are consistent with the fact that none of these sites are found in the sequence of the gene. Finally, the pattern observed after *Msp*I digestion is also consistent with predicted fragments of 2067 and 3870 bp and a larger 5′ fragment extending from nucleotide 3622 to an undetermined *Msp*I site upstream of the gene.

Human β Protein—The human β protein comprises 244 amino acids residues and has a molecular mass of 26,532 Da (Fig. 6). Like rat (243 amino acids) and mouse (236 amino acids) β, human β contains four hydrophobic segments suggestive of transmembrane domains, but no leader peptide. Fig. 7 shows an alignment of the human sequence with the rat and mouse sequences. The consensus sequence for β (data not shown) from the three species (rat, mouse, and human) shows that 91.4% of the amino acid residues are homologous, whereas 68.7% are identical.

Transfection in COS-7 Cells: Expression of Human and Hybrid FcεRI Receptors—We have shown previously that cotransfection of α, β, and γ cDNAs is necessary to promote expression of rat or mouse FcεRI on the surface of transfected COS-7 cells (12). By contrast, cotransfection of human α and γ cDNAs results in the surface expression of αγ complexes without apparent need for β (18). With the availability of human β cDNAs, we analyzed whether human β would influence in any way the efficiency of surface expression of the human receptor complex. Table I shows that cotransfection of human α and γ cDNAs into COS-7 cells resulted in 10.4 ± 8.7% of the cells being fluorescent when analyzed by fluorescence-activated cell sorting after binding of fluoresceinated IgE. This level of expression was not significantly modified when human β cDNA was cotransfected with human α and γ cDNAs (8.3 ± 5.0%). Thus, human β does not seem to influence the level of surface expression of human FcεRI in transfected COS-7 cells. Substituting rat β for human β had a

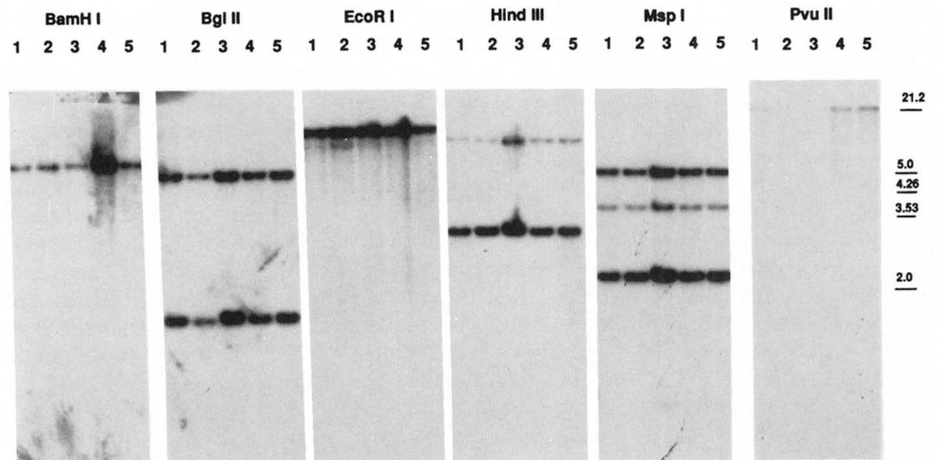

FIG. 6. **Southern blot analysis of genomic DNAs obtained from five different individuals.** The DNAs were subjected to distinct restriction endonuclease digestions, blotted, and hybridized with the human full-length cDNA. The numbers on the top indicate the different individuals, whereas each panel corresponds to a different restriction digest. Size standards are indicated on the right (in kilobases).

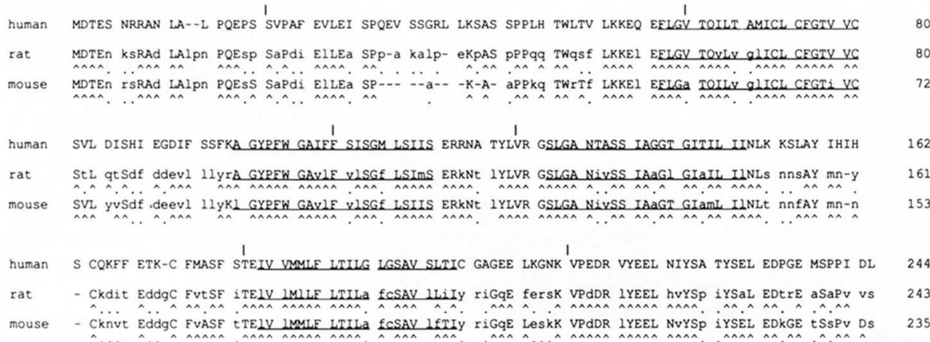

FIG. 7. **Amino acid sequence of human FcεRI β subunit and alignment with rat and mouse β.** Identical and nonidentical amino acid residues are indicated by *upper-* and *lower-case letters*, respectively. The identities and closely related exchanges are indicated by *carets* in the query line, whereas the distantly related exchanges are denoted by *dots*. Nonhomologous exchanges show no marking in the query line. Gaps are indicated by *dashes*. The transmembrane domains are *underlined*, and the splice sites are indicated by *vertical bars*.

TABLE I

Functional expression of FcεRI after transfection of various subunit combinations

Transfected cDNAs			Fluorescent cells (FACS)[a]	
			n	Mean ± S.D.
				%
Human α	—	—	1	0.2
Human α	human β	—	1	0.2
Human α	—	human γ	7	10.4 ± 8.7
Human α	human β	human γ	7	8.3 ± 5.0
Human α	rat β	human γ	4	5.4 ± 3.4
Rat α	rat β	rat γ	8	18.0 ± 17.8
Rat α	human β	rat γ	10	2.4 ± 2.0
Rat α	human β	human γ	5	1.8 ± 1.3
Mouse α	mouse β	mouse γ	4	8.2 ± 5.6
Mouse α	human β	mouse γ	6	1.6 ± 1.2
Mouse α	human β	human γ	2	1.5 ± 0.8
Human α	—	rat γ_{trunc}	7	1.4 ± 1.0
Human α	rat β	rat γ_{trunc}	5	3.2 ± 2.8
Human α	human β	rat γ_{trunc}	7	7.4 ± 7.9
Rat α	rat β	rat γ_{trunc}	2	9.3 ± 0.8
Rat α	human β	rat γ_{trunc}	2	0.4 ± 0.5

[a] FACS, fluorescence-activated cell sorting; trunc, truncated.

tendency to reduce the level of expression (5.4 ± 3.4%), although that difference is not statistically significant.

We then analyzed the effect of substituting human β for rat β. Cotransfection of rat α, β, and γ cDNAs resulted in a much higher level of expression (18.0 ± 17.8%) than cotransfection of rat α and γ with human β (2.5 ± 2.0%) (Student's t statistic, 2.75; $p \leq 0.014$). Similarly, cotransfection of mouse α, β, and γ cDNAs was more efficient (8.2 ± 5.6%) than cotransfection of mouse α and γ with human β (1.6 ± 1.2%) (Student's t statistic, 2.91; $p \leq 0.019$). Since replacing rat γ or mouse γ with human γ did not restore expression (compare 2.4 with 1.8% and 1.6 with 1.5%), it is likely that the problem in expression resides in the human β/rat α or human β/mouse α interaction.

It is known that truncation of the cytoplasmic tail of rat γ prevents the surface expression of human α in transfectants (25). We therefore tested whether human β could complement for the surface expression of human α under these conditions. We first confirmed that cotransfection of human α with truncated rat γ permitted only very poor surface expression of $\alpha\gamma$ complexes (1.4 ± 1.0%). When human β was cotransfected with the latter combination, there was an increase in expression (7.4 ± 7.3%, $n = 7$), although it is not statistically significant. However, this increase did become significant ($p \leq 0.035$) when one aberrant point was not included in the seven experiments. The same increase was not observed when rat β was substituted for human β (3.2 ± 2.8%), suggesting again that there may be specific points of interaction between human α and β. In other experiments using truncated rat γ, we found that human β could not be substituted for rat β in its interaction with rat α (compare 9.3 ± 0.6 with 0.4 ± 0.4%) (Student's t statistic, 13.0; $p \leq 0.006$).

Taken together, these data indicate that there is a tendency for human β to interact more efficiently with human α than does rat β, but the species specificity is weak. By contrast, there is a strong species specificity in the interaction between rat β and α or between mouse β and α.

As shown previously (18) and again here, human $\alpha\gamma$ complexes can be expressed on the surface of transfected cells. Moreover, cotransfection of human α and γ with rat β results in only 20% of the receptors being $\alpha\beta\gamma$ complexes, with the remaining 80% being $\alpha\gamma$ complexes (18). Therefore, it is theoretically possible that $\alpha\gamma$ complexes occur naturally. However, in view of the species specificity of interaction between human β and α (see above), our previous results

obtained from the cotransfection of human α and γ with rat β may not reflect the *in vivo* situation. Nevertheless, it is still possible that a certain proportion of αγ complexes are expressed on the surface of β positive basophils and mast cells. We are presently attempting to generate an antibody specific to human β to address this possibility.

Acknowledgments—We thank Lawrence B. Schwartz for the gift of cDNA libraries and Michael Lerman and Farida Latif (National Cancer Institute, Frederick, MD) for providing genomic Southern blots. We also thank Robert Numerof and Paul Roche for critical reading of the manuscript and Laura Chung for her help in the transfection studies.

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