

Complete Structure of the Mouse Mast Cell Receptor for IgE (Fc ϵ RI) and Surface Expression of Chimeric Receptors (Rat-Mouse-Human) on Transfected Cells*

(Received for publication, April 27, 1989)

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The high affinity receptor for IgE (Fc ϵ RI) found on mast cells and basophils is a tetrameric complex of a single α subunit, a single β subunit, and two identical γ subunits. The genes for the three subunits of mouse Fc ϵ RI have now been cloned from the mast cell line, PT18. When compared at the DNA level, the rat and mouse subunits are similarly conserved. However, at the protein level the homology between mouse and rat α is surprisingly low (71% identities) especially in the cytoplasmic regions (57% identities) which are of different length (25 and 20 residues, respectively). By contrast the β and γ are homogeneously conserved between mouse and rat (83 and 93% identities, respectively). The consensus amino acid sequence of the α subunit derived from three species (rat, mouse, and human) shows that the cytoplasmic tail diverges to the same extent as the leader peptide. Conversely, the transmembrane domain of the α is highly conserved and contains 10 consecutive residues that are identical. Comparisons between mouse Fc ϵ RI and other mouse proteins reveal regions of high homology between the α subunit and Fc γ RIIIa and between the γ subunit and the ζ chain of the T cell receptor.

Cells transfected with the α gene express the α subunit on their surface very inefficiently. Efficient expression is only achieved after co-transfection of the three rodent genes or of the human α gene together with the rodent γ without apparent need for β . The subunits are completely interchangeable upon transfection so that various chimeric mouse-rat-human receptors can be expressed.

The high affinity receptor for IgE (Fc ϵ RI) found on mast cells and basophils is a key molecule in triggering allergic reactions. It is a tetrameric complex of a single IgE-binding α subunit, a single β subunit, and two disulfide-linked γ subunits (1). We and others have isolated the gene coding for the α subunit of the rat receptor (2-5). In addition we have recently cloned the genes for the rat β and γ subunits and expressed the complete receptor by gene transfer (6, 7). Surface expression of IgE binding activity was only achieved upon co-transfection of all three genes. Similarly, the gene for the human α subunit (3, 8) yielded surface expression of

human IgE binding activity when co-transfected with the genes for the rat β and γ (9). Surprisingly, human IgE binding activity was also detected after co-transfection of the genes for the human α and rat γ , i.e. without an apparent need for a β subunit (9). In order to confirm these results of co-transfection in a different system and to compare rigorously the sequences of Fc ϵ RI subunits with the products of other mouse genes (especially homologous Fc γ Rs), we have cloned the three mouse genes from the non-neoplastic cell line, PT18. Here we report the isolation of these genes and describe their requirements for expression and their ability to assemble into receptors when combined with subunits from other species.

EXPERIMENTAL PROCEDURES¹

RESULTS

Cloning and Sequencing of the α , β , and γ Subunits of Mouse Fc ϵ RI—A library prepared from mRNA of the nonmalignant mast cell line PT18 was screened with labeled fragments of the rat α , β , and γ cDNAs. After three rounds of screening, a number of positive clones were isolated and purified; three clones were identified with the α probe, two clones with the β probe, and four clones with the γ probe. For each group we sequenced at least two independent clones completely, at least one on both strands. The cDNA and the deduced amino acid sequences corresponding to the α , β , and γ subunits of mouse Fc ϵ RI are presented in Figs. 1, 2, and 3, respectively. Hydrophobicity plots of the subunits of mouse Fc ϵ RI were similar to those for rat Fc ϵ RI and showed hydrophobic segments suggestive of a leader peptide and one transmembrane domain in both α and γ and of four transmembrane domains in β (data not shown). The complete primary structure of mouse Fc ϵ RI is presented in Fig. 4 in the form of a model recently proposed for rat Fc ϵ RI. In the model for mouse Fc ϵ RI the nonconserved residues between rat and mouse are shaded so that the most conserved regions can be clearly seen.

Comparison of the α , β , and γ Subunits of Fc ϵ RI in Different Species—The rat, mouse, and human α cDNAs and the rodent β and γ cDNAs were compared by using the GAP program. Similarity scores were derived from both the translated and untranslated sequences so that possible differences could be identified. The results are shown in Table I. Overall, the DNA sequences for the α , β , and γ subunits are similarly conserved between rat and mouse, and the conservation of the γ subunit,

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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) J05018, J05019, and J05020.

¹ Portions of this paper (including "Experimental Procedures," Figs. 1-3 and 5-8, and Table III) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

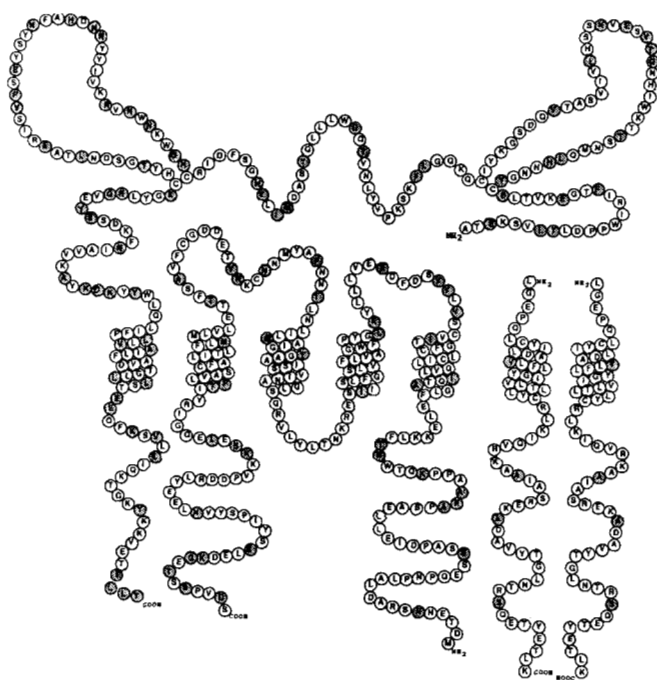


FIG. 4. Model of the mouse high affinity IgE receptor. The extracellular portion of the processed α subunit is shown at the top and the remainder of the chain on the left. To the right of the α subunit is the β subunit with its four transmembrane segments, and to the right of the β subunit is the dimer of γ subunits. The putative transmembrane segments were determined by the method of Engelman *et al.* (14) and shown as consisting of 21 residues. The single letter code for amino acids is used (15). The nonconserved residues between rat and mouse are shaded.

TABLE I
Comparisons between species: the subunits of FcεRI

Subunits	Species	Identity scores		
		DNA		Protein
		Untranslated	Translated	
α	Mouse/rat	88	83	70
	Mouse/human	68	69	48
	Human/rat	68	69	45
β	Mouse/rat	87	89	80
γ	Mouse/rat	84	94	93

but not of the other subunits, is higher in the translated sequence (94% versus 84%).

The amino acid sequences of the three subunits were also compared. The identity scores are shown in Table I. The consensus sequences derived from the LINEUP program are presented in Figs. 5–7 for the α , β , and γ subunits, respectively. When compared to mouse β , the rat β showed an insert of 8 residues which could be the result of an additional exon (Fig. 6). However, the β subunits are highly conserved between rat and mouse as are also the γ subunits (80 and 93% identities, respectively). By contrast, only 70% identical residues are found between the rat and mouse α subunits. In order to identify possible clusters of conserved residues, regions of the α subunit were compared in the three species. Table II shows that the leader peptide and the intracellular domain are the least conserved and the transmembrane domain the most conserved region. This is true when the mouse and rat α are compared and is even more prominent when the consensus sequence from three species is analyzed. About 38% of the

TABLE II
Comparisons between species: % identical residues in α subunit regions
LP, leader peptide; EC, extracellular region; TM, transmembrane domain; IC, cytoplasmic tail.

Species	LP	EC	TM	IC	Total
Mouse/rat	65	71	76	57	70
Mouse/human	28	51	71	16	48
Human/rat	18	50	67	29	45
Consensus	17	42	62	16	38

residues are identical in the entire sequence of the three species. The percentage of identical residues in the extracellular region (42%) is similar to the overall value but in the leader peptide and intracellular domains (17 and 16%, respectively) is only half as high whereas in the transmembrane domain (62%) it is almost twice as high. In addition the predicted lengths of the intracellular segments are substantially different in the three species: 20 residues in the rat, 25 in the mouse, and 31 in the human.

Comparison of Mouse FcεRI with Other Mouse Receptors—The mouse α of FcεRI is homologous to all mouse FcγRs but particularly to FcγRIIa (16). Table III shows that the two immunoglobulin-related domains are conserved between the two receptors suggesting that gene duplication from a common ancestor occurred during evolution. However, the most conserved region lies in the transmembrane domain where 8 consecutive residues are identical. Conversely very little homology is seen in the cytoplasmic domains.

No significant homology was found between the mouse β of FcεRI and other mouse proteins. By contrast the mouse γ of FcεRI was found to be substantially homologous to the ζ chain of the T cell receptor (17). As can be seen in Fig. 8, the 29 N-terminal residues of the γ share 55% identical residues with the corresponding segment of the ζ chain.

Surface Expression of FcεRI in Transfected Cells—In order to study the requirements for surface expression of the IgE binding α subunit, the genes for the subunits of the receptor from different species were subcloned independently into the expression vector pSVL, and various combinations of these genes were transfected into COS-7 cells. Expression of IgE binding activity on the surface of transfected cells was assessed by an IgE rosetting assay and by FACS² analysis (Table IV). When transfected alone, the human, rat, or mouse α induces a small number of rosetting cells, a number well below the limit of sensitivity of the FACS analysis. No rosettes were ever observed with untransfected COS-7 cells or with cells transfected with β and/or γ or when transfected cells were preincubated with a 20 $\times$ molar excess of nonspecific IgE. This underscores that the background associated with the rosetting assay as used in our studies is essentially zero so that the sensitivity of this single cell assay can be completely exploited.

No apparent increase in the efficiency of expression was seen when rodent α was cotransfected with the rodent β or γ . By contrast, cotransfection of human α with rodent γ but not with β resulted in a more than 50-fold increase in the efficiency of surface expression (2.3–5.2% by rosetting assay, 4.4–5.6% by FACS analysis). Comparable results were obtained when human α was cotransfected with both β and γ suggesting that β alone cannot promote the surface expression of human α . However, in the rodent system, efficient expression is achieved only after cotransfection of the three subunits (4.9–6.5% by rosetting assay, 4.6–7.8% by FACS analysis) without apparent statistical difference between a particular combina-

² The abbreviation used is: FACS, fluorescence activated cell sorter.

TABLE IV

Surface expression of FcεRI after gene transfer

Values are means ± S.D.

Transfected genes			IgE rosette assays		FACS analysis	
Mouse	Rat	Human	Number of rosettes /total cells		% fluorescent cells	
α---	---	-	2/39,000	n = 3	<0.10	n = 2
---	α---	-	1/45,000	n = 3	<0.10	n = 2
---	---	α	3/44,000	n = 4	<0.10	n = 2
αβ-	---	-	1/10,000	n = 1	ND ^a	
-β-	---	α	2/8,000	n = 1	ND	
---	-β-	α	2/18,000	n = 2	ND	
α-γ	---	-	2/10,000	n = 1	ND	
---	α-γ	-	1/8,000	n = 1	ND	
-β-γ	---	-	0/30,000	n = 2	<0.10	n = 2
			% rosetting cells			
-β-γ	---	α	2.35 ± 0.90 n = 2		5.65 ± 1.15 n = 2	
---	-β-γ	α	5.20 ± 3.15 n = 9		4.40 ± 0.65 n = 2	
αβγ	---	-	6.50 ± 3.50 n = 2		7.80 ± 4.75 n = 2	
αβ-	-β-γ	-	3.75 ± 1.75 n = 2		4.95 ± 1.95 n = 2	
α--	-βγ	-	0.70 ± 0.15 n = 2		2.20 ± 0.20 n = 2	
α-γ	-β-	-	1.60 ± 1.25 n = 2		3.35 ± 1.15 n = 2	
---	αβγ	-	4.90 ± 2.20 n = 12		4.60 ± 0.05 n = 2	
-βγ	αβ-	-	4.35 ± 0.20 n = 2		4.95 ± 1.50 n = 2	
-βγ	α--	-	1.85 ± 0.20 n = 2		3.70 ± 0.90 n = 2	
-β-	α-γ	-	2.35 ± 0.20 n = 2		3.45 ± 0.20 n = 2	
---	-βγ	α	4.70 ± 2.60 n = 18		ND	

^a ND, not determined.

tion of mouse and rat genes. Finally, where it is possible to compare them, the results from the rosetting assay correlated well with those obtained from the FACS analysis.

DISCUSSION

Although the molecular mechanisms for signal transduction mediated by FcεRI are still unknown, it is reasonable to postulate that whatever they are will likely be similar in different species. Moreover, the molecular structures responsible for these functions are likely to be more conserved through evolution than the less critical portions of the molecule. In this regard, the cloning of the mouse FcεRI has generated unpredictable observations; the sequences and the lengths of the cytoplasmic tail of the α subunit diverge in three species to the same extent as the leader peptide (Table II) (Fig. 5). By contrast the transmembrane domains are highly conserved and contain a stretch of 9 consecutive residues that are identical. This suggests a functional role for the transmembrane domain of the α subunit either by itself or by interaction with the β and the γ subunits or still undefined molecules. Similarly, the pronounced conservation between mouse and rat β and particularly γ also suggests a functional importance for these subunits (Table I). The model shown in Fig. 4 helps to identify the most conserved regions of these subunits, for example the cytoplasmic loop between the second and third transmembrane domains of β.

The cloning of mouse FcεRI allowed us for the first time to compare the sequences of mouse α, β, and γ with other mouse proteins, particularly with the homologous FcγRs which are chromosomally linked to the α (18). The most striking homologies are found in the transmembrane domains of FcγRIIa where the same 8 residues that are conserved in the transmembrane domain of α (see above) are also found in FcγRIIa (Table III). It is plausible that the transmembrane domains of both receptors serve a similar function (19). The recent finding of the association of FcγRIIa with a subunit identical to the γ subunit of FcεRI is consistent with that interpreta-

tion.³ Interestingly, the γ subunit of FcεRI shares 55% identical residues with the mouse ζ subunit of the T cell receptor (Ref. 9 and Fig. 8) as well as other striking similarities; both form disulfide-linked dimers, both exhibit very short extracellular segments (5 and 8 residues for γ and ζ, respectively), and both have a single transmembrane domain with a common aspartic acid. The common localization of the genes for those subunits on mouse chromosome 1⁴ together with the described similarities suggests a common origin. Possibly gene duplication occurred to form a family of functionally related proteins. Obviously, a possible relationship between the γ and ζ remains to be explored.

From the results of the experiments of transfection and by analogy with other system (20, 21) we postulate that a "filtering system" prevents unassembled receptors from reaching the plasma membrane. This would explain why co-transfection of rodent α, β, and γ is necessary for the surface expression of the rodent receptor. Occasionally the α subunit by itself would bypass the filtering system to reach the surface. This may indeed happen in every transfected cell but be quantitatively undetectable in most of the cells with the assays used in this study. That a few rosetting cells are observed in these conditions would then be due to an impaired filtering system in these particular cells. Many questions remain. What is the filtering system? Why is the β required for the surface expression of the rodent but not of the human α? Are the differential requirements due to the mixing of species? Do human α(γ)_n complexes normally exist? Obviously, it will be interesting to address some of these questions when human β and γ will be available.

The complete interchangeability of the subunits between species shown in the experiments of co-transfection will prevent us from studying the function of individual subunits from the rat by transfecting mouse mast cells or vice versa. However, studies on the function of human α should now be feasible by transfecting rodent mast cells.

Acknowledgments—We wish to thank Drs. William Gause and Toru Takashi for help in the FACS analysis, Gary Smithers for assistance in the computer analysis, and Dr. Henry Metzger for helpful discussions.

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Complete structure of the mouse mast cell receptor for IgE (FcεRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells

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cdNA cloning, screening and sequencing

Total RNAs and poly(A)⁺ mRNAs from the mouse mast cell line P18 were prepared by guanidium isothiocyanate extraction, cesium chloride gradient and separation on oligodT column as before (2). Double stranded cDNAs were prepared, treated with T4 DNA polymerase and with Eco RI methylase, and ligated to Eco RI linkers (New England Biolabs, Beverly, MA) as described previously (2). The cDNA library was constructed by inserting the cDNA into the vector lambdaBZAP (Stratagene, La Jolla, CA) that had been previously digested with Eco RI and treated with calf intestine alkaline phosphatase (Boehringer Mannheim, Germany). The ligated DNAs were packaged with Giga Pack (Stratagene, La Jolla, CA) and the phage library was amplified in *E. coli* HB4 was coinfected with the lambdaBZAP library and with F1 helper phage, allowing for the automatic excision, recircularisation, and packaging of the plasmid script SK phagids containing the cloned inserts. Once the phagids were amplified, the library was titrated by the cloned treatment and the library was propagated in *E. coli* strain XL-1.

The library was screened as previously described (2,6) with the following nucleic acid labeled cDNA probes: the Sty^r fragment (bp 149-810) from the rat α cDNA, the two Eco RI-Pst I fragments (bp 1477 and 788-1150) from the rat β cDNA and the full length rat γ cDNA. Oligonucleotides corresponding to the sequence of rat α (bp 58-87) and to the sequence of rat β (bp 37) were synthesized in 36 nucleotide long fragments (Pharmacia LKB, Foster City, CA) labeled at the 5'-end and used to identify full length clones when appropriate. Hybridization of the nitrocellulose filters with cDNA probes were performed at 42°C in 4X SSC; subsequent washes were done twice at room temperature in 2X SSC and twice at 55°C in 2X SSC. Washing was done in 2X SSC once at 55°C and three times at 45°C. After three rounds of screening two to four independent positive clones were selected for each gene and the cDNAs sequenced directly from the T3 and T7 promoters by the dideoxy termination method using Sequenase as before (6,7). The cDNAs were sequenced without subcloning using oligonucleotide primers synthesized as above.

Sequence comparisons

Comparisons between mouse, human and rat sequences were performed using the GAP and the LINEUP program (12)

Experiments of transfection

COS-7 cells (American Type Culture Collection, Rockville, MD - CRL 1651) were grown in Dulbecco's modified Eagle medium with 2 mM glutamine, 16% FCS, 10 U/mL penicillin, 10 µg/mL streptomycin and 60 µg/mL tylosine (Gibco, Gaithersburg, MD) at 37°C in 5% CO₂/95% air.

The following DNA fragments were subcloned separately into the Sma I site of the SV40 promoter-driven expression vector pSPV (PharMacia, Upsala, Sweden): the Eco RI-Pst I fragment (bp 1 to 852), the full length sequence, and the Eco RI-Nhe I fragment (bp 1 to 388) from the mouse α , β , and γ cDNAs respectively. Various combinations of these constructs (total amount of DNA 80 μ g per 0.8×10^6 cells) were transfected in COS-7 cells by electroporation (1000 V, 10 μ s, 1 pulse) and the cells were harvested 24 h after 48 h after transfection. After transfection the surface expression of Fc γ RI was assessed by using an IgE rosetting assay and by FACS analysis. The IgE rosetting assay was performed either as before (7,9) by mixing transfected cells with TNP-coated erythrocytes that had been preincubated with anti-DNP IgE, either by mixing transfected cells with TNP-coated erythrocytes that had been preincubated with anti-Fc γ RI IgE. For the FACS analysis, cells that had been transfected were incubated for 1 hour at room temperature in phosphate buffered saline containing 0.1 μ MBSA and 0.1 μ M NaN₃ with 2.5-5 μ g/ml FITC conjugated monoclonal anti-DNP mouse IgG (H1-DNP-25-86) prepared as before (13). The negative control cells were incubated with 0.1 μ M of mouse or human IgG. The cells were then rinsed on the spin column, the chain of the transfected cells. The cells were then rinsed once in the buffer, fixed in phosphate buffered saline containing 2.5 μ M paraformaldehyde and kept at 4 °C. The analysis was performed on a FACSCAN (Becton Dickinson, Mountain View, CA) using a 488 nm wavelength and a 15 mW argon-ion laser with a band pass filter of 530 nm. The data was analyzed using the FACS software on a 486 3000 computer using the Becton Dickinson FACSCAN and Consort 30 software.

																								ACGTCCT				8										
GGTCAGCTTAGCACTGGAAGGTGACAGGGGGC																								ATG	CTG	ACT	GGA	AGC	TCT	GCC	CAG	CTG	TGC	CTA	GCA	LA	LA	-12
																								M	V	T	G	A	R	S	A	Q	L	C	L	A		
CTG	CTG	TTG	ATC	TCT	CTT	GAT	GTC	ATT	CTA	GCC	ACT	GAG	AAA	TCT	GTA	CTG	ATC	TTG	L	135	9																	
L	L	F	M	S	L	D	V	I	L	A	T	E	K	S	V	L	L	-1																				
GAC	CCA	CCA	TGG	ATT	AGA	ATA	TTT	ACA	GGA	AAA	GTG	ACC	TCT	TCC	TGC	TAT	GGG	AAC	195	29																		
D	P	P	W	H	I	R	I	F	T	G	E	K	V	A	S	C	Y																					
AAT	CAC	CTT	CAA	ATG	ACA	TCT	ACT	ACT	AAA	TGG	ATC	CAC	AAT	GGT	ACC	TCT	TCT	GAG	GTG	255	49																	
N	H	L	L	Q	M	N	S	T	T	K	M	I	H	N	G	T	V	S	E	V																		
AAC	TCT	TCA	ATT	CTG	GTC	ATT	GTG	AGT	GCC	ACG	GTT	CAA	AGT	GGA	AAA	TAC	ATA	TCG	315	69																		
N	S	S	H	L	V	I	V	S	A	T	V	Q	D	S	G	K	Y	I	C																			
CAG	AAG	CAA	GGA	TTG	TTT	AGC	ATA	CTG	CTG	TTC	TTC	ATG	ATG	ACG	CAA	GAT	TGG	CTG	375	89																		
Q	K	Q	C	G	L	F	K	S	K	P	V	Y	L	N	V	T	Q	D	W	L																		
CTC	CTC	CAG	ACA	TCT	GCT	GAC	ATG	CTA	TTA	CTC	CAT	GGA	TCC	TTT	GAT	AGA	TGC	CAT	435	109																		
L	L	Q	T	S	A	D	M	I	L	L	V	H	G	S	F	D	R	C	H																			
GGC	TGG	AAG	CAA	TGG	ATA	GTC	CGC	AGC	ATG	ATC	TAC	TAC	AGG	AAT	GAC	CAT	GTC	TTC	AAC	495	129																	
G	W	K	N	K	M	N	V	N	R	K	V	I	Y	Y	R	N	D	H	A	F																		
TAC	AGT	TAT	GAC	AGC	CCC	GTC	TCC	ATT	AGA	GAC	GCC	ACG	CTG	AAT	GAG	GGC	ACC	TAC	Y	555	149																	
T	S	Y	E	S	P	V	S	I	R	E	A	T	L	N	D	S	G	T	Y																			
CAC	TGC	AAG	GCG	TAT	CTT	AGG	CAG	GGA	TAA	GAA	TCT	GAA	AAA	TTC	AGA	ATT	GCT	GTA	615	159																		
H	C	K	G	Y	L	R	Q	V	E	Y	E	S	D	S	K	F	R	I	A	V																		
GTA	AAA	GCT	TAC	NAAG	TGC	AGG	TAT	TGG	CTA	CAA	ATA	ATT	TTC	CCA	TTG	TTG	GGC			675																		
V	K	A	Y	K	C	K	Y	Y	W	L	Q	L	I	F	P	L	L	V	A	189																		
ATT	CTT	TTT	GCT	GTC	GAC	ACG	GCG	TTA	TTG	CTC	TCA	ACA	GAA	CAG	CTG	AAA	TCA	CTA	735	209																		
L	L	F	A	Y	V	D	T	G	L	L	L	S	T	E	E	Q	F	K	S	V																		
TTG	GAG	ATT	CAG	AAG	ACT	GGA	AAA	TAC	AGG	AAA	GTT	GAA	ACG	GAA	CTC	CTA	ACG	ATA	795	229																		
L	E	L	Q	X	K	T	G	K	K	K	K	V	E	E	T	E	L	T	L																			
CATATAGGAACCTAATGCTCATTTGCTGGAAGAAGCACTTTCAACGAGTAATTTTTCGAGAAATCTCCAAATAGCCTTCCTACCT																								815														
CTGAAGAGGCCACTCATGTGCAAGACAGAAAGCTTGTCCACAGCAATGCTCAATTAAGTCTTCATTAAAGGCTCCAGC																								875														
TGGTATGTAGTCAATGCAATTAAGCAATATTCATTAATCAACTGAGAAAGAA																								904														

Figure 1 Nucleotide sequence and predicted amino-acid sequence of the mouse FcRI α subunit. The putative transmembrane region is underlined. Residues 5' to residue +1 encode a putative signal peptide.

[illegible]

Figure 2 Nucleotide sequence and predicted amino-acid sequence of the mouse FcγRI β subunit. The four putative transmembrane regions are underlined.

TGATGCCAGCTCCAGCGCGGAGCCGCCAGCGGACCCAGG ATG 45
 M -18
 ATC TCA GCC GTG ATC TTG TTG CTT TTG GTG GAA CAA GCA GCC GCC CTG GGA GAG 105
 I S A V I L F L L L V E Q A A A L G E 3
 CCG CAG CTC TGC TAT ATC CTG GAT GCT GTC CTG TTT TTG TAT GGT ATT GTC CTT ACC CTA 165
 P Q L C Y I L D A V L F F L Y G I V L T L 23
 CTC TAC TGT CGA CTC AAG ATC CAG GTC CGA AAG GCA GCT ATA GCC AGC CGT GAG AAA GCA 225
 L Y C R L K I Q V R K C A A I A S R E K A 43
 GAT GCT GTC TAC ACG GGC CTG AAC CGG AGC CAG GAG ACA TAT GAG ACT CTG AAG CAT 285
 D A V Y T G L N T R S Q E T Y E T L K H 63
 GAA AAA CCA CCC CAG TAG CTTCAGAACAGATGTACTTGGTGATCTTTTCACTCTTAATCTCTCCGAG 358
 E K P P Q - 68
 CCCCTTGGTCACCTCTGTGCTTTGAAGCATCACATAATGATGCTAGCTAGGCTCATACAGTGTACATGGCAGGTC 437
 CCCATCTCCGTTAAAGACTTACTCAGTACATTTCTCTTCCAGGCTCTTGGCTTCTTTCTCTTCTCTCCCTGA 516
 TCCTCGACTCTCATAACAAATGGAAGGAGTATCCCAATAAAGCTGCGAGAGACTGAAAAAAGAAAAA 533

Figure 3 Nucleotide sequence and predicted amino-acid sequence of the mouse FcεRI γ subunit. The putative transmembrane region is underlined. Residues 5' to residue +1 encode a putative signal peptide.

1 50
 Mouse NVTGR SAQLCLALLF NELDVLITAT EKVLTLDPP WIRIFTGKEV
 Rat NDTGG SARLCIALVL ISLGVMLTAT QKVVSLLDP WIRILTQDRV
 Human MKKAPAMES PTLICVALLF PAPDGLAVF QKPKVSLNPF WNKIFKGENV
 Consensus m.tg. sa.LCLALLf .slav.LtAt qKvvsLDPP WIRIFTGkhv
 51 100
 Mouse TLSCYGNMHL QNNSTTKMIR NGTVSEVMS HLIVISATVQ DSGRYICQKQ
 Rat TLICGNMNS QNNS.TKMIH NDSISNVMS HWIVSATIQ DSGRYICQKQ
 Human TLICGNMNF EVSS.TWPH NDSISNVMS HLIVISATVQ DSGRYICQKQ
 Consensus TL.CNGNM... qnns.TKMIH Ngs.Sev.sS hliVIsAt.q DSGRYICQKQ
 101 150
 Mouse GLFKSKPVYL NVTDQWLLQ TSADNVLVHC SFDIRCHGWK NNVKRVIIY
 Rat GFYKSKPVYL NVNDEWLLQ SSADVLDNG SFDIRCHGWK NNVKRVIIY
 Human QVNESEPVYL EVFSDWLLQ ASAEVWEGQ FLPLRCHGWK NNDVIRVIY
 Consensus g..kSkPVYL nV.qdWLLQ .SADVvl..g sfdirChgwK nN.V.RVIY
 151 200
 Mouse RNDHAPNYSY ES.PVSIREA TLNDSGTYHC KGYLRQVEYE SDFPRIAVVK
 Rat RDDIAFKYSY DNNHISIRKA TPNDSGTYHC TGYLNRVECK SDFPSIAVVK
 Human RDKGALKYMY ENNHISITHA TPNDSGTYHC TGYVWLDYH SDFPLNITVIR
 Consensus kdd.AKYSY es.nisir.A T.ndSGTYHC tGyl.qveye Sdkf.IaVVK
 201 250
 Mouse AFREKYWLQ LIFFLIVAIL FAVDTGLLS TEROPESVLE IQRTGKVKK
 Rat DYTYEYRWLQ LIFFSLAVIL FAVDTGLMFS TEROPESVLE IQRTGKVKK
 Human AFREKYWLQ FFIPLVILV FAVDTGLFS TQOQVFLFK IRRTKGFRL
 Consensus ay..kY.WLQ liFFlLvVil FAVDTGL..S T..Qf.s.Lk IqRTgKghk
 251 262
 Mouse VETELLT
 Rat VET
 Human LNFHFKPNPK NN
 Consensus Vet.....

Fig. 5 Alignment of Mouse, Rat, and Human α from FcεRI. The alignment was performed by using the GAF program and the consensus sequence was derived with the LINEUP program as described in Experimental Procedures. In the consensus sequence a capital letter indicates a perfect match between all three species, a lower case letter indicates a match between two of the three species, and a dot indicates no match.

1 50
 Mouse RDTENKSRAD LALPNQRES SAPDIELLEA SPARAA..... PPKQTN
 Rat RDTENKSRAD LALPNQRES SAPDIELLEA SPARALPER PABPPQQTW
 Consensus RDTEN.SRAD LALPNQRES. SAPDIELLEA SP...A.....PP.QTW
 51 100
 Mouse RFLKKELEF LGATQILVGL ICLCFQIVC SVLYVSDPDE EVLLLYKLG
 Rat QFLKKELEF LGVTVLVGL ICLCFQIVC SELQTSDDO EVLLLYKAGI
 Consensus ..FLKKELEF LG.TQ.LVGL ICLCFQIVC S.L...SDPD. EVLLLY..GY
 101 150
 Mouse PFWGAVLVVL SGFLSIIRER ENTLYLVRS LGANIVSSIA AGGIAHLIL
 Rat PFWGAVLVVL SGFLSIIRER ENTLYLVRS LGANIVSSIA AGGIAHLIL
 Consensus PFWGAVLVVL SGFLSI.SER ENTLYLVRS LGANIVSSIA AG.GIA.LIL
 151 200
 Mouse NLNHNAYNN NCMVTEDDG CFVASTTEL VLMFLFLIL AFCSAVLFTI
 Rat NLNHNAYNN YCKDITDDG CFVASTTEL VLMFLFLIL AFCSAVLFTI
 Consensus NL.NN.AYNN .CK..TEDDG CFV.SP.TEL VLM.LFLIL AFCSAVL...I
 201 243
 Mouse YRIQKELESF KVFDDRLYE LVNYSPIYS LEDRGETSS VDS
 Rat YRIQKELESF KVFDDRLYE LVNYSPIYS LEDRGETSS VDS
 Consensus YRIQKE... KVFDDRLYE L.VNYSPIYS. LED..E.S.P V.S

Fig. 6 Comparison of Mouse β with Rat β from FcεRI. The alignment and the consensus sequence were performed as in Fig. 5.

1 50
 Mouse NISAVILFLL LLVEQAALG EPOLCYILDA VLFLYGIYLT LLYCRLEKQ
 Rat NISAVILFLL LLVEQAALG EPOLCYILDA VLFLYGIYLT LLYCRLEKQ
 Consensus NI.AVILFLL LLVE.AAALG EPOLCYILDA .LFLYGIYLT LLYCRLEKQ
 51 86
 Mouse KKAASREK ADAYVTGLNT RQSTYETLK HEKFPQ
 Rat KKAASREK ADAYVTGLNT RQSTYETLK HEKFPQ
 Consensus KKA.IASREK .DAYVTGLNT R.QSTYETLK HEKFPQ

Fig. 7 Comparison of Mouse γ with Rat γ from FcεRI. The alignment and the consensus sequence were performed as in Fig. 5.

Gamma LGEPQLCYIL DAVFLYGIY LTLLYCRLEK
 Zeta LLDPLCYLL DGLIFGIYV ITALYLRAR
 Consensus L..P.LCY.L D..LP.YG...T.LY.R.K

Fig. 8 Alignment of the γ subunit (residues 19 to 47) from Mouse FcεRI and of the ζ subunit (residues 26 to 54) from the Mouse T cell receptor. The alignment and the consensus sequence were performed as in Fig. 5.

Table III
 COMPARISONS BETWEEN THE α SUBUNIT OF MOUSE FcεRI AND MOUSE FcγRIIA

	Number of residues		% IDENTITY SCORES
	α SUBUNIT	FcγRIIA	
Leader Peptide	23	30	0
Extracellular	181	185	35
Ig Domain 1	49	48	45
Ig Domain 2	49	50	34
Transmembrane	21	21	48
Intracellular	25	26	0.04
Processed peptide	227	231	33
Unprocessed peptide	250	261	29