Genomic organization of mouse Fc_γ receptor genes

(Fc receptors/exon–intron junctions/protein domains/immunoglobulin gene superfamily)

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ABSTRACT We have isolated and characterized the gene coding for the mouse Fc receptor that is termed Fc_γRIIa. The gene contains five exons and spans approximately 9 kilobases. Unlike most members of the immunoglobulin gene superfamily, this gene utilizes multiple exons to encode its leader peptide. The first exon encodes the hydrophobic region of the signal sequence; the second exon, which contains only 21 base pairs, encodes a segment of the signal peptidase recognition site; and the beginning of the third exon encodes the predicted site of peptidase cleavage. The third and fourth exons each code for immunoglobulin-like extracellular domains. The fifth exon encodes the hydrophobic transmembrane domain and the cytoplasmic tail. Partial characterization of the Fc_γRIIb gene indicates that it also contains multiple leader exons, including a 21-base-pair exon and two exons coding for homologous immunoglobulin-like extracellular domains. However, the Fc_γRIIb gene uses four exons to encode its intracytoplasmic region. Analysis using contour-clamped homogeneous electric field (CHEF) gels indicates that the Fc_γRIIa and Fc_γRIIb genes are linked within 160 kilobases on mouse chromosome 1.

Fc receptors are a family of cell surface proteins that bind to the Fc regions of immunoglobulins and thereby enable antigen–antibody interactions to influence cellular responses (for reviews, see refs. 1 and 2). Interestingly, not only can distinctive Fc receptor genes be expressed on different cell types, but also different spliced messages from the same Fc receptor gene can be expressed in a tissue-specific manner (1–6). The result of antigen binding to antibody molecules that are bound to different Fc receptors is also dependent upon cell type (1, 2)—e.g., ingestion and processing of antigen in macrophages (6), regulation of antibody synthesis in B cells, and triggering of histamine release from mast cells.

Mouse macrophages express three distinct types (1) of Fc receptors specific for IgG (Fc_γR): one (Fc_γRI) that specifically binds monomeric IgG2a; a second (Fc_γRII) that binds aggregated IgG1, IgG2a, and IgG2b (7, 8); and a third Fc_γRIII that binds only the minor subclass, IgG3. Two different but homologous cDNAs (Fc_γRIIa and Fc_γRIIb) each encode receptors for aggregated IgG (2–5). The gene encoding Fc_γRIIa is expressed only in macrophages (3, 4), and its expression is selectively induced by γ interferon (6). The gene encoding Fc_γRIIb is expressed in both macrophages and lymphocytes, with alternative splicing producing two transcripts, b₁ and b₂, which differ only in their cytoplasmic regions (3–5). The genes for Fc_γR that have been cloned (Fc_γRIIa and Fc_γRIIb genes) are genetically inseparable from the *Mls-1* locus on mouse chromosome 1 (9–11). The *Mls-1* locus controls B-cell products that stimulate mixed lymphocyte reactions in *H-2*-compatible strains and, with the class II major histocompatibility complex molecule I-E, are

responsible for intrathymic deletion of a large fraction of potentially self-reactive T-cell clones (11–13).

An understanding of the relationship of the various Fc receptor genes to each other, the factors influencing their evolution, and molecular mechanisms regulating their expression requires a detailed analysis of the structures of their genes. Here we report the characterization of two members of this gene family.

MATERIALS AND METHODS

Screening of Mouse cDNA and Genomic Libraries. A NZW mouse thymus cDNA library in phage λgt10 was screened by using the ³²P-end-labeled oligonucleotide probes 5'-GGA-CCTGGCTCCGGATGGACCTCCCATTGTGGAAC-CACTG-3' and 5'-GAAATAAAGGCCCGTGTCCTGCTG-CAAACAGGAGGCACATC-3', which correspond to Fc_γRIIb1 cDNA and Fc_γRIIa cDNA (nucleotides 544–583 and 723–762, respectively) (3). Two NZW cDNA clones were isolated. One clone, "a," contained an insert of 0.8 kilobase (kb) specific for Fc_γRIIa starting at "Fc_γRα" nucleotide 706 (3). A second clone, "b," contained a 1.5-kb insert that corresponds to "Fc_γRβ1," starting at nucleotide 273 and including 510 nucleotides with 96.7% identity between Fc_γRIIb and Fc_γRIIa (3).

The 1.5-kb cDNA insert of clone b was labeled with ³²P by the random priming method (14) and was used to screen two genomic libraries: a mouse BALB/c library in EMBL-3 (Clontech), and a mouse BALB/c library in the cosmid vector pTCF (15). Screening, hybridization, and washing procedures were carried out as described (16–18). Isolated colonies were rescreened with an Fc_γRIIa-specific probe (a 0.42-kb *Bgl* I fragment of the cDNA insert of clone a).

Initial screening of 500,000 plaques of the BALB/c genomic library in EMBL-3 yielded three independent clones containing exons 4 and 5 of the Fc_γRIIa gene (see Fig. 1) and three independent clones containing portions of the Fc_γRIIb gene (see Fig. 3). From a BALB/c genomic cosmid library of 300,000 colonies, four independent overlapping cosmid clones were isolated, and three of these clones contained all five exons of the Fc_γRIIa gene.

Characterization of Genomic Clones. DNAs from phage and cosmid clones and specific subcloned fragments were characterized by standard restriction endonuclease mapping and Southern blot analyses. DNA sequencing of specific restriction fragments subcloned into pBluescript SK vectors (Stratagene) was performed by using the chain-termination sequencing method. Coding regions and exon–intron junctions were sequenced in both directions by using T3 and T7 promoter primers of pBluescript SK and synthetic oligonucleotides (20-mers) corresponding to known exon or intron

FIG. 1. Structure of the mouse *Fc γ RIIa* gene. Exons are numbered and shown as boxes. All restriction sites for *Bam*HI (B), *Eco*RI (R), and *Hind*III (H) are indicated. The four subclones used for mapping and sequencing are labeled by their restriction site boundaries and size. Exon 1 is represented from the start of translation.

sequences. Nucleotide sequences were analyzed by using the MicroGenie program (Beckman).

Contour-Clamped Homogeneous Electric Field (CHEF) Gels. Mouse J774 DNA was prepared in low-gelling-temperature agarose as described (19), and aliquots were digested with restriction enzymes. Samples were electrophoresed in 1% agarose gels with a CHEF hexagonal array apparatus (20) for 28 hr. Field direction was reoriented by using a switch ramp from 0.3 to 15 sec with the field strength at 6 V/cm. Gels were hybridized at 65°C with ³²P-labeled *Fc γ RIIb*-specific (*Apa* I/*Bgl* I) or *Fc γ RIIa*-specific cDNA probes and were washed with 0.5× SSC at 65°C (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7).

RESULTS

Organization and Sequence of the Mouse Gene Encoding *Fc γ RIIa*. A restriction map derived from cosmid clones of genomic DNA containing the *Fc γ RIIa* gene is shown in Fig. 1. The *Fc γ RIIa* gene consists of five exons distributed over ≈9 kb. The exon-intron boundaries and all coding regions of the *Fc γ RIIa* gene were sequenced (Table 1). All introns conform to the GT-AG rule (21), and surrounding sequences are closely related to the consensus sequences surrounding splice junctions (22). Exon 1 encodes the 5' noncoding region and most of the leader peptide. Exon 2 is an unusually small 21-base-pair (bp) exon encoding amino acids that form part of the signal peptidase recognition site (23, 24). The 5' end of exon 3 also participates in coding for the amino acids bordering the signal peptidase cleavage site [Fig. 2, middle (*Fc γ RIIa*) sequences]. Exons 3 and 4 each encode a single extracellular domain with the characteristics of the immunoglobulin superfamily (26). Exon 5 encodes a small extracel-

FIG. 2. Comparison of the 21-bp exons of the *Fc γ RIIb*, *Fc γ RIIa*, and *Fc ϵ R* α -chain genes and the segments of signal sequence that they encode. Nucleotide homologies are indicated in boxes. Amino acids in parentheses are partially encoded by the 21-bp exons, whereas bracketed amino acids are encoded by the adjacent exon. Arrows indicate preferred predicted sites of peptidase cleavage for *Fc γ RIIb* (3–5), *Fc γ RIIa* (see text), and *Fc ϵ R* α -chain (25) gene products.

lular segment, the transmembrane and cytoplasmic regions, and the 3' noncoding region. The exons range in size from 21 bp (exon 2) to 648 bp (exon 5). All nucleotides in the coding region for mouse *Fc γ RIIa* match the published cDNA clone (3).

Introns range in size from 0.66 kb (intron A) and 0.85 kb (intron B) to 3.5 kb (intron C). The shortest introns flank the smallest exon. The poly(A) signal AATAAA (27) is found in exon 5 (nucleotides 1307–1312).

Partial Map of the Gene Encoding *Fc γ RIIb*. In screening the BALB/c genomic library in EMBL-3, we isolated three clones that contained portions of the *Fc γ RIIb* gene. A partial map of the *Fc γ RIIb* gene was constructed from these clones (Fig. 3) and includes six of the exons. The *Fc γ RIIb* gene, like the *Fc γ RIIa* gene, contains a 21-bp exon that encodes a portion of the leader peptide required for the peptidase cleavage site [Fig. 2, top (*Fc γ RIIb*) sequences; also denoted L* in Fig. 3]. The *Fc γ RIIb* gene also contains two exons that encode immunoglobulin-like extracellular domains (EC1 and EC2). Unlike the *Fc γ RIIa* gene, the *Fc γ RIIb* gene contains three exons coding only for cytoplasmic segments of the receptor (C1, C2, and C3). The alternative splicing event previously described for *Fc γ RIIb* (3) involves elimination of the 141-nucleotide C1 segment. The exon-intron boundaries thus far determined are shown in Table 2. Based on cDNA sequence (3–5), at least two additional exons must be present, one or more to encode the remainder of the leader peptide and at least one to encode the transmembrane portion with short adjacent intracytoplasmic and extracellular segments (TM).

Restriction fragments from genomic DNA that were detected by various ³²P-labeled *Fc γ RII* cDNA probes in South-

Table 1. Exon-intron junctions of the mouse *Fc γ RIIa* gene

Exon			Intron		
No.	Size, bp	5' splice donor	Designation	Size, kb	3' splice acceptor
1	130*	CTGTTTG ¹³⁰ <u>gtgagt</u> LeuPheA	A	0.66	tcttcttttacag CTTTTCGA laPheAla
2	21	CAGAGTG ¹⁵¹ <u>gtaagt</u> GlnSerA	B	0.85	tctcactctcag CAGCT ↓ CTT laAla ↓ Leu
3	258	ATTTCTG ⁴⁰⁹ <u>gttagt</u> IleSerA	C	≈3.5	tattgccttcag ACTGGCTG spTrpLeu
4	255	GTCCAAG ⁶⁶⁴ <u>gtgagc</u> ValGlnA	D	≈2.1	cctcccttcag ATCCAGCA spProAla
5	648	TCCTATAATAAA ¹³¹²			

Exon sequences are shown in upper case letters; intron sequences are shown in lower case letters. Nucleotides bordering introns are numbered according to ref. 3. The predicted site of peptidase cleavage is indicated by an arrow.

*Size of the longest known transcribed portion of exon 1 starting with nucleotide 1 (3).

FIG. 3. Partial map of the mouse $Fc\gamma RIIb$ gene. Exons are shown as boxes. L* indicates a 21-bp exon that encodes a portion of the leader peptide, EC1 and EC2 denote exons coding for immunoglobulin-like extracellular domains, TM indicates an as yet uncloned presumed exon encoding the transmembrane region, and C1, C2, and C3 represent exons coding from cytoplasmic regions of the receptor. Restriction enzyme sites for *Bam*HI (B), *Eco*RI (R), and *Hind*III (H) are indicated. The three subclones used for mapping and sequencing are labeled. Only the translated portion of exon C3 is shown.

ern blots (Fig. 4 and ref. 4) could be accounted for by the $Fc\gamma RIIa$ and $Fc\gamma RIIb$ gene maps. This suggests that these loci exist as single copies in the mouse genome.

Linkage of $Fc\gamma RIIa$ and $Fc\gamma RIIb$ Genes. By using CHEF gels and $Fc\gamma RIIa$ -specific and $Fc\gamma RIIb$ -specific probes, both of the genes were localized to the same *Nar*I fragment and *Sfi*I partial digest fragment, each ≈ 200 kb in size, and to the same ≈ 160 -kb *Nar*I/*Sfi*I fragment (data not shown). Specificity is established because these genes were localized to different *Xho*I fragments.

DISCUSSION

Gene Organization and Putative Protein Domains. The structure and organization of the mouse $Fc\gamma RIIa$ and $Fc\gamma RIIb$ genes have been investigated in the present study. The $Fc\gamma RIIa$ gene consists of five exons contained within 9 kb (Fig. 1 and Table 1). The $Fc\gamma RIIb$ gene has a more complex organization that includes at least eight exons (Fig. 3 and Table 2). Exons 3 and 4 of the $Fc\gamma RIIa$ gene and the corresponding exons of the $Fc\gamma RIIb$ gene (EC1 and EC2) encode the extracellular domains of 85 or 86 amino acids, which are homologous to other members of the immunoglobulin supergene family. Each extracellular domain of both Fc receptor genes is encoded by a separate exon, which is a common characteristic of this supergene family (26). The nucleotide homology between $Fc\gamma RIIa$ gene exon 3 (258 bp) and the corresponding $Fc\gamma RIIb$ gene EC1 exon (255 bp) is 95.7%. Also, the $Fc\gamma RIIa$ gene exon 4 (255 bp) is extremely homologous to the corresponding $Fc\gamma RIIb$ gene EC2 exon

FIG. 4. Southern blot analysis of $Fc\gamma RII$ genes. C57BL/6 mouse liver DNA was digested with *Eco*RI (lane 1) or *Hind*III (lane 2) and probed with the 1.5-kb insert of clone b ($Fc\gamma RIIb$ cDNA). Molecular size markers are indicated. The $Fc\gamma RIIa$ gene accounts for the 10.1-kb *Eco*RI band (exons 2–5) and the 6.0-kb *Hind*III band (exons 3–4) of the genomic Southern blot. The $Fc\gamma RIIb$ gene structure accounts for the 1.4-kb *Eco*RI band (exon EC1), and the remaining ≈ 3 -kb and ≈ 9 -kb *Eco*RI bands presumably represent the fragment containing the EC2 exon and the fragment containing the cytoplasmic exons, respectively.

(255 bp) (97.6% nucleotide identity). This marked homology between extracellular domains is consistent with the observations that both receptors demonstrate similar specificities in binding to Fc regions of IgG (6). In contrast, the membrane-spanning and intracytoplasmic domains of these receptors lack homology (3). Furthermore, the portions of the genes encoding these domains are markedly different in organization—the $Fc\gamma RIIa$ gene utilizes only one exon (exon 5) to encode the entire transmembrane–cytoplasmic region, whereas the $Fc\gamma RIIb$ gene uses at least four exons. In this region the lack of homology and the differences in genomic structure suggest that these receptors may transduce signals and function intracellularly in very different ways.

The genomic organization of the mouse $Fc\gamma RIIa$ gene is strikingly similar to the organization of the rat $Fc\epsilon RI$ α -chain gene, which contains five analogous exons (28). Also, it has been noted that mouse $Fc\gamma RIIa$ and rat $Fc\epsilon RI$ α -chain share an identical eight-amino acid transmembrane sequence (25)

Table 2. Partial exon–intron organization of the mouse $Fc\gamma RIIb$ gene

Exon		5' splice donor	Intron size, kb	3' splice acceptor
Designation	Size, bp			
(L')	(—)	GTGCTAA ⁴¹²	—	cctttcttacag ATCTTGCT
L*	21	↓ ACTCATG ⁴³³ gtaagt	≈ 1.2	aaaattgagcag ATCTTCCA
EC1	255	ATTCTCTG ⁶⁸⁸ gtagt	≈ 1.4	ctttcag ATCTTCCA
EC2	255	GTCCAAG ⁹⁴³ gtgagc	—	GGCCCAAG
(TM)	(123)	GTTCAG ¹⁰⁶⁶	—	tgccctcctag CTCTCCC
C1	141	AGCCCAT ¹²⁰⁷ gtgagt	0.75	tttcatccacag ACAATCCT
C2	38	ACTGAG ¹²⁴⁵ gtgagg	0.10	tgctttccctag GCTGAG
C3		ATTTAG ¹³³²	—	

Exon sequences are shown in upper case letters; intron sequences are shown in lower case letters. Nucleotides bordering introns are numbered according to Ravetch *et al.* (3) as amended (4). Parentheses and dashes indicate gaps in genomic organization. Only the translated portion of exon C3 is shown. The predicted site of peptidase cleavage (3–5) is indicated by an arrow.

and associate with the same M_r 10,000 subunit (29). Together, these observations suggest that $Fc_\gamma RIIa$ and $Fc_\epsilon RI$ α chain (but not $Fc_\gamma RIIB$) might share functional similarities or associate with similar components. Like the $Fc_\gamma RIIa$ and $Fc_\epsilon RI$ α -chain genes, the genes encoding the T-cell receptor α chain, the major histocompatibility complex class II α chain, and the MRC OX-2 glycoproteins utilize only one exon to encode their entire transmembrane and cytoplasmic region (26).

As a rule, members of the immunoglobulin supergene family encode their signal sequences by using a single exon (26). The first exceptions to this rule were the mouse and human CD3 γ chains, which utilize one exon to encode the hydrophobic region of the leader peptide and another to encode the signal peptidase cleavage site (30, 31). The $Fc_\epsilon RI$ α -chain gene (28) and the $Fc_\gamma RIIa$ and $Fc_\gamma RIIB$ genes now represent additional exceptions in the immunoglobulin gene superfamily to the "signal peptide-single exon" rule. These three related genes each contain an unusually small conserved exon (21 bp) that encodes the amino acids of the leader peptide that are predicted to form part of the peptidase-recognition site and to contain or to border the peptidase cleavage site (Fig. 2). Based on statistical prediction methods (23, 24), we predict that peptidase cleavage of $Fc_\gamma RIIa$ occurs between Ala-31 and Leu-32 (Fig. 2), rather than the site previously predicted (3). Thus, in the $Fc_\gamma RIIa$ gene, the first three exons are predicted to encode the signal sequence. The 21-bp exons of the three Fc receptor genes exhibit considerable nucleotide homology, but little amino acid homology, and different predicted peptidase cleavage sites (Fig. 2).

The exon-intron boundaries of the $Fc_\gamma RIIa$ and $Fc_\gamma RIIB$ genes are quite similar. For example, the 5' splice donor sites of $Fc_\gamma RIIa$ gene introns B, C, and D (Table 1) are identical to the homologous donor sites of the $Fc_\gamma RIIB$ gene (Table 2).

It has been recognized that mouse $Fc_\gamma RIIa$ and $Fc_\gamma RIIB$ are homologous to human $Fc_\gamma RII$ (32–34) and human $Fc_\gamma RIIB$ (35, 36), particularly in their extracellular domains. Comparison of mouse $Fc_\gamma RIIa$ gene exons 2, 3, and 4 with the corresponding regions of human $Fc_\gamma RII$ gene (nucleotides 91–111, 112–369, and 370–624 of ref. 33) shows 76%, 72%, and 73% nucleotide homology, respectively. Although exon 5 of $Fc_\gamma RIIa$ gene has little homology, the C2 and presumed TM exons of $Fc_\gamma RIIB$ gene (Fig. 3) demonstrate 66% and 71% homology, respectively, with corresponding regions of human $Fc_\gamma RII$ gene (32–34). Also, the ends of the human $Fc_\gamma RII$ intron "remnant," CTTCTGgtcagt...tgtgtcttcagAGTG-GCTG (34), where lower case letters signify intron sequences, are remarkably similar to the corresponding mouse $Fc_\gamma RIIa$ intron (intron C of Table 1), which suggests that exon-intron boundaries may also be conserved across species in this gene family.

Evolution and Linkage of $Fc_\gamma R$ Genes. The similarities in exon structures and the 96–98% nucleotide homology between exons encoding extracellular domains indicate that the $Fc_\gamma RIIa$ and $Fc_\gamma RIIB$ genes are products of gene duplication. This conclusion is supported by the demonstration that both genes are physically linked on the same 160-kb genomic DNA fragment. The duplication of the two extracellular exons within each gene must have occurred much earlier than the duplication to form two genes because the nucleotide homology between exons 3 and 4 of the $Fc_\gamma RIIa$ gene is 44.2% and homology between $Fc_\gamma RIIB$ exons EC1 and EC2 is 49.3%. The extraordinary homology between genes suggests that gene duplication has been a relatively recent event and/or that strong selection pressures have been exerted on these receptors to conserve these structures.

The close proximity of these genes should facilitate mapping of this important region of mouse chromosome 1. Mapping may be useful in localizing the gene locus that encodes the *Mls-1^a* product, which has been inseparable from the $Fc_\gamma RII$ genes in genetic studies (9–11).

Note Added in Proof. We have found transcriptional startpoints of the $Fc_\gamma RIIa$ gene to be at nucleotides 1 and 37 (Table 1) by using RNase protection and S1 nuclease assays. Regarding the biological consequences of alternative mRNA splicing events, it has recently been shown that the C1 exon of the $Fc_\gamma RIIB$ gene encodes a region that prevents accumulation of the receptor in clathrin-coated pits (37). Also, analogous to the 21 nucleotide exons that we describe (Fig. 2), the human $Fc_\gamma RIIB$ gene contains a 21-nucleotide exon, and a transcript lacking it has been found (38). We suggest that alternative splicing that deletes transcription of this 21-nucleotide "mini-exon" may produce "proreceptors" that do not undergo peptidase cleavage but have N termini tethered to the membrane until an alternate proteolytic event.

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