

RECEPTORS FOR Fc ϵ AND Fc γ ARE LINKED ON MOUSE CHROMOSOME 1

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Recently isolated cDNA clones for the high affinity Fc ϵ receptors on mast cells and basophils (Fc ϵ RI α) and Fc γ receptors on macrophages and lymphocytes (Fc γ 2b/ γ 1R) are homologous members of the Ig supergene family. Analysis of the segregation of restriction fragment length polymorphism in crosses of inbred mice now establish that the structural genes encoding both Fc ϵ RI α and Fc γ 2b/ γ 1R are indeed discrete genes and are linked at the distal end of mouse chromosome 1. This finding raises the possibility that a family of Fc receptors could be found in a region that is known to contain immunologically important markers of lymphocyte surface Ag and autoimmune defects.

Among recently cloned FcR, the poly IgR (1), Fc γ 2b/ γ 1R (2-5) and Fc ϵ RI α ² (6, 7) all share common Ig-like domains and belong to the Ig supergene family (8). The conserved homology among members of this family suggest they are derived from a common evolutionary precursor. However, gene mapping studies have demonstrated that members of this family are not clustered on any one particular chromosome in eukaryotes but are widely dispersed throughout the genome.

Extensive gene mapping studies in the mouse have linked cDNA probes encoding Fc γ 2b/ γ 1R (α , β 1, β 2) to the *Ly-17* alloantigen locus on distal chromosome 1 (2-4). Although it appears that *Ly-17* may be expressed by an Fc γ 2b/ γ 1R gene, it remains to be determined which Fc γ 2b/ γ 1R cDNA, in fact, encodes the bona fide *Ly-17* surface Ag.³ For the purpose of this paper it will be assumed that Fc γ 2b/ γ 1R and *Ly-17* are genetically indistinguishable (2-4, 9, 10).

In this report, we establish that a cDNA probe encoding the structural gene for the α -subunit of the high affinity

Fc ϵ R hybridizes to a unique set of RF⁴ in the mouse which do not overlap with Fc γ 2b/ γ 1R. The locus of Fc ϵ RI α is also linked to the macrophage receptor Fc γ 2b/ γ 1R on chromosome 1 by virtue of the distribution of mouse inbred alleles among genetic recombinants. In a separate genetic backcross experiment we demonstrate that the genes encoding Fc ϵ RI α and Fc γ 2b/ γ 1R are linked to an established marker of chromosome 1 α -spectrin (*Spna-1*).

MATERIALS AND METHODS

Mice. Inbred or recombinant inbred strains of mice were acquired from the following sources: BALB/cJ, C57BL/6J, C57L/J, C58/J, NZB/B1NJ, and SWR/J (The Jackson Laboratory, Bar Harbor, ME), AKR/N, BALB/cAn, C57BL/10N, DBA/2N, SJL/JLwPt (Hazleton Laboratory, Rockville, MD), AKR/FuRdA $\times$ 020/A (OXA) and BALB/cHeA $\times$ STS/A(CXS) (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The (C57BL/10ScSn $\times$ SWR/J)F₁ $\times$ SWR/J backcross mice were generously supplied by J. Blackwell (London School of Hygiene and Tropical Medicine, London, England).

Hybridization probes. The cDNA probe for Fc ϵ RI α is a 600 bp *StyI* restriction fragment that encodes nearly the entire coding sequence of the α -subunit from rat basophilic leukemia cells (6). The Fc γ 2b/ γ 1R α probe, generously provided by J. Ravetch (Memorial Sloan Kettering Cancer Center, New York, NY) is a 1.3-kb insert from the mouse J774 cDNA clone (3). The *Spna-1* probe is a 750-bp *PstI* insert from the mouse α spectrin cDNA clone pMaspl¹ generously supplied by P. Curtis (The Wistar Institute of Anatomy and Biology, Philadelphia, PA) (11). The *Ren-1.2* probe is a 1.4-kb *PstI* insert of the mouse cDNA clone *Id-2* kindly provided by K. Gross (Roswell Park Memorial Institute, Buffalo, NY) (12).

Preparation of DNA and Southern hybridization. DNA was made from liver or spleen preparations of strains of inbred mice (13). The DNA was restricted with the appropriate restriction endonuclease and subjected to 0.7% agarose gel electrophoresis in 40 mM Tris-acetate 20 mM sodium acetate, 1 mM EDTA pH 7.6. In preparation for Southern transfer, the agarose gel was treated as described (13) and subsequently transferred to Nytran filters. Hybridization conditions are as reported (13) and final wash stringency for all hybridizations in this study was 65°C at 0.2 $\times$ SSC, (1 $\times$ SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) 0.1% SDS, 5 mM EDTA for 30 min.

Genetic crosses and recombination frequency. The CXS recombinant inbred series consist of 14 strains inbred for more than 30 generations from which the inheritance pattern of more than 75 polymorphic loci have been established (14). Similarly, the OXA recombinant inbred series consists of 14 strains from which the positions of more than 27 loci have been established (J. Hilgers, unpublished data). Estimates of gene order and genetic distance were calculated using the method of Silver and Buckler (15). The recombination frequencies were generated from cumulative totals of both panels.

RESULTS AND DISCUSSION

Fc ϵ RI α hybridizes to single polymorphic gene distinct from Fc γ 2b/ γ 1R α . Genomic DNA from a panel of inbred

⁴ Abbreviation used in this paper: RF, restriction fragment; LP, length polymorphism.

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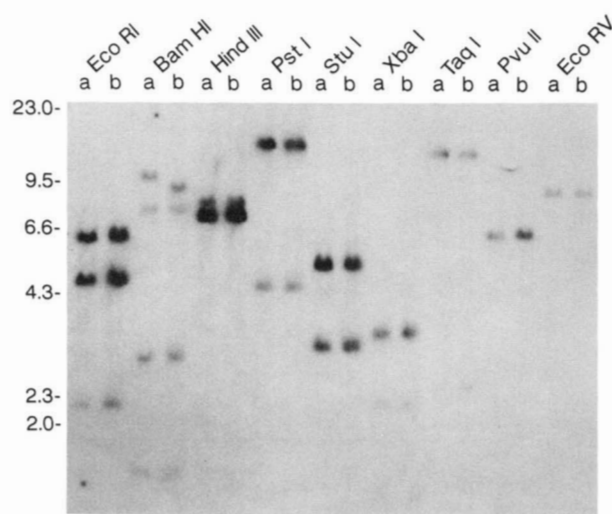
² There has been some confusion in the literature about the use of Greek letters with regard to the nomenclature of FcR: The " α " of the Fc ϵ RI refers to the α -subunit of the high affinity R which binds to IgE (8). The " α ", β 1, and β 2" of the Fc γ 2b/ γ 1R cDNA probes refer to analogous genes that code presumably for different Fc γ R (2-4).

³ The expression patterns and chromosomal location of *Ly-17* has been determined by using the 2.4G2 mAb (9, 10). The Fc γ 2b/ γ 1R cDNA probes, however, do not show consistent mRNA expression patterns with the tissue distribution of 2.4G2 (2-4).

mice BALB/c and DBA/2N was digested with restriction endonucleases and hybridized to a 600 bp *Sty*-1 RF of *FcεRIα* cDNA which spans almost the entire coding region (Fig 1). The *FcεRIα* probe appears to hybridize to a single *EcoRV* RF band of 9.5 kb confirming that *FcεRIα* is most likely found as a single copy gene (7). A *Bam*HI RFLP was detected between BALB/c and DBA/2N (Fig. 1) and a third *Bam*HI RF pattern was observed when a more extensive panel of inbred mice was examined (Fig. 2). As a result of the *Bam*HI RFLP, we could assign haplotypes for *FcεRIα* among inbred mice (Table I).

The same panel of inbred mice also generated three *Bam*HI RF patterns with the probe *Fcγ2b/γ1RIα* which were different from the patterns observed with *FcεRIα* (Fig. 3). Furthermore, the distribution of the haplotypes also differs between *Fcγ2b/γ1RIα* and *FcεRIα* (Table I). For example, C57 L/J and DBA/2N carry *Fcγ2b/γ1RI^a* and *Fcγ2b/γ1RI^c* haplotypes, respectively, whereas both strains uniformly carry on *FcεRIα^b* pattern. In addition, comparison of published RF patterns observed by using *Fcγ2b/γ1RIα* and *Fcγ2b/γ1RIβ* probes (2-4) showed no correspondence with RF patterns found in Figure 1. Thus, the probes for *FcεRIα* and *Fcγ2b/γ1RI* appear to recognize separate genes.

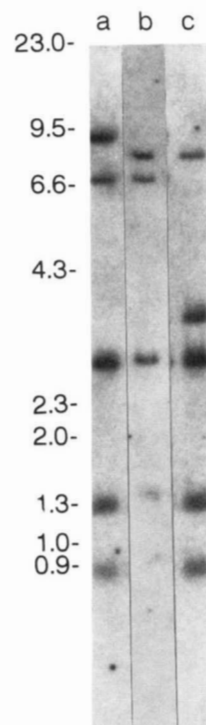
The significance of the allelic distribution of the *FcR* genes remains to be determined but provides an immediate opportunity to genetically map *FcεRIα* in the mouse by determining the frequency of genetic recombination in a Mendelian population of recombinant inbred mice (16). The polymorphism detected between inbred mice BALB/cHeA and STS/A by digestion with *Bam*HI and probing with *FcεRIα* allowed us to examine a panel of 14 recombinant inbred mice generated between these parental strains to determine possible linkage with previously mapped chromosomal loci (14) (Fig. 4). By comparison of the strain distribution pattern with known loci and using standard statistical methods for linkage analysis of recombinant inbreds (15), *FcεRIα* shows significant con-



FcεRIα Probe

Figure 1. Southern hybridization of *FcεRIα* to mouse genomic DNA. High m.w. genomic DNA from two inbred mice BALB/cAn (a) and DBA/2N (b) was digested with the restriction endonucleases listed, electrophoresed on 0.7% agarose gels, and transferred to Nytran filters as in *Materials and Methods*. A ³²P-labeled 600 bp *Sty*I fragment isolated from a full length cDNA clone of *FcεRIα* (6) was hybridized to the filters under conditions described (13).

Inbred Strain Haplotypes



FcεRIα Probe-BamHI Digestion

Figure 2. Inbred strain haplotypes defined by a *FcεRIα* *Bam*HI RFLP. Electrophoresis, hybridization probe, conditions, and stringencies are as in Figure 1. Representative *FcεRIα* *Bam*HI patterns a, b, and c are labeled above their respective lanes.

TABLE I
Inbred Strain Haplotypes^a

Inbred Strain	<i>Fcγ2b/γ1RIα</i>	<i>FcεRIα</i>
AKR/N	ND	a
BALB/c (An, HeA, J)	a	a
C57BL/6J	b	b
C57BL/10N	b	b
C57L/J	a	b
C58/J	ND	c
DBA/2N	c	b
NZB/BINJ	ND	a
020/A	ND	b
SJL/JLw	ND	a
STS/A	ND	c
SWR/J	a	c

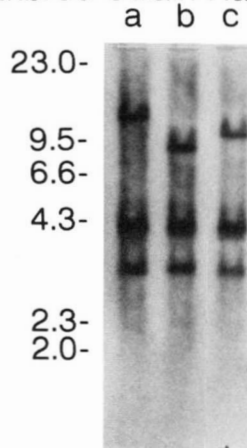
^a Haplotype patterns are generated by *Bam*HI digestion and hybridization with probes for *Fcγ2b/γ1RIα* and *FcεRIα*.

cordance with several loci that have been established on the distal end of mouse chromosome 1 (Table II). Specifically, *FcεRIα* shared the strain distribution pattern with alkaline phosphatase (*Akp-1*), *Mtv-7* (14), *Ly-17* (9), and *Fcγ2b/γ1RIα* (17) in 13 of 14 strains.

A similar panel of 14 recombinant inbred mice derived from offspring of 020/A and AKR/FuRdA parental strains were similarly examined (Table II). Genetic concordance between *FcεRIα* and *Ly-17* (12/14) and *Akp-1* (12/13) independently confirms the positioning of *FcεRIα* to mouse chromosome 1.

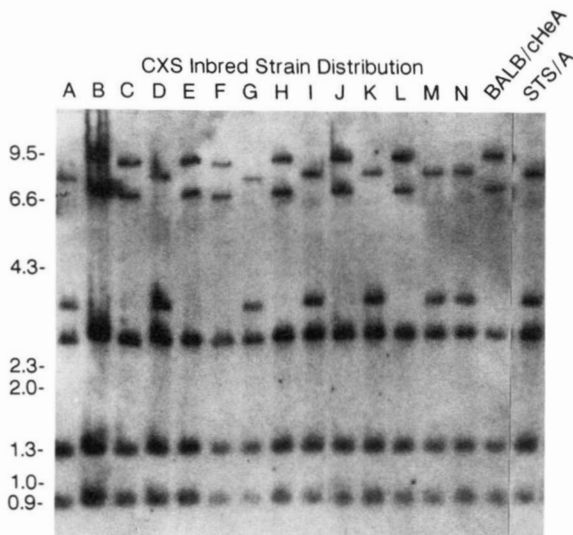
To refine the genetic distance between *Fcγ2b/γ1RI* (*Ly-17*) and *FcεRIα* we also examined a panel of (C57BL/10 ScSn × SWR/J)_{F1} × SWR/J mice for frequency of genetic recombination. After following the segregation of *Bam*HI RFLP, no recombinations were detected between *Fcγ2b/γ1RIα* and *FcεRIα* among 40 backcross mice (Table III).

Inbred Strain Haplotypes



Fcγ2b/γ1Rα Probe-
Bam H1 Digestion

Figure 3. Inbred strain haplotypes defined by an *Fcγ2b/γ1Rα* BamH1 RFLP. Electrophoresis, hybridization conditions, and stringencies are as in Figure 1. The *Fcγ2b/γ1Rα* probe is a 1.3 kb *Pst*I insert from the cDNA clone J774 (3).



FcεRIα Probe-BamH1 Digestion

Figure 4. *FcεRIα* southern hybridization analysis of CXS recombinant inbred mice. BamH1 restricted DNA from 14 CXS recombinant inbred strains (A to N) was hybridized to *FcεRIα* as in Figure 1. The parental strains, BALB/c HeA and STS/A, are presented on the right-hand side for comparison.

TABLE III

Analysis of recombination frequency in (C57BL/10 ScSn × SWR/J) F1 × SWR/J backcross mice^a

Breeders	Loci:	Ren 1,2	<i>Fcγ2b/γ1Rα</i>	<i>FcεRIα</i>	<i>Spna-1</i>	n
SWR/J		S	S	S	S	
C57BL/10 ScSn		B	B	B	B	
(B10 × SWR/J)F1		S/B	S/B	S/B	S/B	
Backcross progeny		S/S	S/S	S/S	S/S	14
F ₁ × SWR/J		S/B	S/B	S/B	S/B	17
		S/S	S/B	S/B	S/B	5
		S/B	S/S	S/S	S/S	4

^a B = C57BL/10 ScSn; S = SWR/J.

Other loci have been mapped to this region of mouse chromosome 1 in the same panel of backcross mice, namely α -spectrin (*Spna-1*) and renin-1,2 (*Ren-1,2*) (18). We also do not detect recombinants between *Fcγ2b/γ1Rα*, *FcεRIα*, and *Spna-1* which indicates close linkage but still prohibits precise positioning of these loci. In contrast, nine recombinants were observed between these loci and *Ren-1,2* (18).

A gene order can be predicted, however, on the basis of the segregation distribution pattern of several recombinants listed in Table II. Specifically, two recombinants (CXSH, OXAI) carry identical alleles for *FcεRIα* and *Akp-1* and two recombinants (CXSJ, OXAE) carry identical alleles for *Fcγ2b/γ1Rα* (*Ly-17*) and *FcεRIα*. This suggests that *FcεRIα* lies between *Fcγ2b/γ1Rα* (*Ly-17*) and *Akp-1*. Inasmuch as *Ly-17* and *Akp-1* have been positioned on chromosome 1 (9, 17, 19, 20), we can predict the following gene order: centromere--*Fcγ2b/γ1Rα* (*Ly-17*) --*FcεRIα*--*Akp-1*.

CONCLUSION

The high affinity *FcεR* and the *FcγR* are found to be linked on the distal end of mouse chromosome 1. This region also harbors other loci of immunologic importance such as the lymphocyte activation locus, *Mls*, lymphocyte surface Ag loci (*Ly-9*, *Ly-22*) and autoimmune markers *gld* and *Ctl* (10, 21, 22).

It is conceivable that additional *FcR* genes may be found in this region of chromosome 1. Linkage of *FcR* genes implies not only a common evolutionary origin but also a possible selection to maintain these genes within close proximity. By using probes from the most conserved regions of homology, additional *FcR* genes may ultimately be isolated and linked by chromosomal gene walking. Sequence comparisons of cDNA clones corresponding to the α , β 1, and β 2 genes of the mouse *Fcγ2b/γ1R* indicate that alternative splicing may result in different expres-

TABLE II

Distribution of *FcεRIα* and *Fcγ2b/γ1Rα* genes with associated loci of mouse chromosome 1^a

Locus	Chromosome	BALB/cHeA × STS/A (CXS) recombinant inbreds													
		A	B	C	D	E	F	G	H	I	J	K	L	M	N
<i>Ly-5</i>	1	S	S	S	S	C	S	S	S	S	C	S	C	C	S
<i>Ly-22</i>	1	S	C	C	S	C	S	S	S	S	C	S	C	S	S
<i>Fcγ2b/γ1Rα</i>	1	S	C	C	S	C	C	S	S	S	C	S	C	S	S
<i>Ly-17</i>	1	S	C	C	S	C	C	S	S	S	C	S	C	S	S
<i>FcεRIα</i>	1	S	C	C	S	C	C	S	C	S	C	S	C	S	S
<i>Mtv-7</i>	1	S	C	S	S	C	C	S	C	S	C	S	C	S	S
<i>Akp-1</i>	1	S	C	C	S	C	C	S	C	S	S	S	C	S	S
020/A × AKR/FuRdA (OXA) recombinant inbreds															
<i>Ly-17</i>	1	O	A	O	A	A	A	A	A	O	A	O	O	O	O
<i>Mls</i>	1	O	A	O	A	A	A	A	A	A	A	O	O	O	O
<i>FcεRIα</i>	1	O	O	O	A	A	A	A	A	A	A	O	O	O	O
<i>Akp-1</i>	1	O	A	O	A	O	A	A	A	A	A	O	O	O	O
<i>Ly-9.2</i>	1	O	ND	O	A	A	A	O	A	A	A	O	O	ND	O

^a STS/A = S, BALB/cHeA = C, AKR/FuRdA = A, 020/A = O.

sion patterns of Fc γ R among hematopoietic cell lineages (3). However, the high affinity Fc ϵ R is exclusively found on mast cells and basophils (6). A better understanding of the organization of the FcR genes in this region of chromosome 1 will lead to further insight into the tissue specific regulation of expression of these genes and to potential interaction of these genes with other immunologic markers.

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