

Fc RECEPTORS

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Abstract

Recent advances in the structural analysis of the genes and proteins for immunoglobulin Fc domain receptors have provided a molecular characterization of this complex family. The wide cellular distribution of these receptors and their functional heterogeneity are reflected in the diversity of molecules which bind antibody and immune complexes. The detailed analysis of the IgG and IgE Fc receptors has indicated that these molecules have evolved from a common precursor through gene duplication. Similarities among these receptors, in both structure and function have emerged. Thus, the Fc receptors provide an example of a class of molecules in which conserved domains are combined with divergent sequences to yield a diversity of function.

INTRODUCTION

The interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody-dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All these interactions are initiated through the binding of the Fc domain of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. It is now well established that the diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of Fc

receptors. Considerable progress has been made in the last three years in defining this heterogeneity for IgG and IgE Fc receptors (Fc γ R, Fc ϵ R) through their molecular cloning. Those studies make it apparent that Fc receptors share structurally related ligand binding domains, but differ in their transmembrane and intracellular domains which presumably mediate intracellular signalling. Thus, specific Fc γ Rs on different cells mediate different cellular responses upon interaction with an immune complex. The structural analysis of the Fc γ Rs and Fc ϵ RI has also revealed at least one common subunit among some of these receptors.

In this review we focus on the characterization of the recently cloned FcRs: the Fc γ Rs and the high affinity Fc ϵ R as well as their associated subunits. In rodent and human systems where our knowledge of these receptors is reasonably complete, analysis of the genes for these receptors has suggested a possible evolutionary relationship. We also review the correlation of structure and function for these receptors. We do not discuss the receptors involved in transport of immunoglobulin, which are structurally distinct from the receptors discussed here. Similarly, the low affinity receptor for IgE (Fc ϵ RII, CD23) is structurally unrelated to the high affinity Fc ϵ R and the Fc γ Rs and has been recently reviewed (1). Finally, systems in which our knowledge is only fragmentary or for which the molecular characterization has not been published, such as Fc receptors for murine IgG3, IgM, or IgA, are not discussed here. For discussions of those topics, the reader is referred to recent reviews (2–4).

NOMENCLATURE

The complexity of the FcRs for IgG and IgE require that a consistent, nontrivial nomenclature be proposed to facilitate the discussion of these molecules. We have attempted to incorporate the recommendations of previous nomenclature committees and, where possible, to conform to the standards established by the WHO.

FcRs are defined by their specificity for immunoglobulin isotypes. Fc receptors for IgG are referred to as Fc γ R, for IgE as Fc ϵ R, for IgA as Fc α R, etc. Structurally distinct receptors are distinguished by a Roman numeral, based on historical precedent. We now recognize three groups of Fc γ Rs, designated *Fc γ RI*, *Fc γ RII*, and *Fc γ RIII*. Two groups of Fc ϵ R have been defined; these are referred to as Fc ϵ RI and Fc ϵ RII. Structurally related although distinct genes within a group are denoted by A, B, C . . . , and where alleles are known they are designated by a superscript, e.g. A^{HR}. Alternative spliced transcripts derived from a single gene are designated a1, a2 . . . , b1, b2, . . . etc. Finally, the protein subunit is given a Greek letter, such as Fc γ RIIIA α , Fc γ RIIIA γ .

Table 1 lists the best known Fc γ R and Fc ϵ RI and their associated subunits, alternative names, monoclonal antibody specificities, chromosomal locations and binding specificity. Previous names are indicated for each of these receptors. A diagrammatic representation of these receptors is presented in Figure 1. Fc γ Rs defined in rodent and human include the high affinity Fc γ RI and the low affinity Fc γ RII and Fc γ RIII. Fc γ RII is encoded by a single gene in the mouse and by three homologous genes—Fc γ RIIA, Fc γ RIIB, and Fc γ RIIC—in the human. Alternative spliced transcripts that encode proteins with identical extracellular and transmembrane domains but distinctive cytoplasmic domains have been identified for Fc γ RIIB in both mouse and human and are referred to as b1, b2, and b3. Fc γ RIII is encoded by a single gene in the mouse and two genes in the human: IIIA and IIIB. Discrete protein subunits are associated with IIIA in both mouse and human and are designated IIIA α , IIIA γ , and IIIA ζ . IIIB encodes a GPI-linked protein molecule. The Fc ϵ R for high affinity binding in mouse, rat, and human is Fc ϵ RI and is composed of three subunits: Fc ϵ RI α , Fc ϵ RI β , and Fc ϵ RI γ . Fc ϵ RII is a low affinity receptor, structurally homologous to lectin-binding proteins.

Fc γ R

An overview of the molecular heterogeneity of Fc γ Rs can be seen in Figure 1, which illustrates schematically the proteins as predicted from molecular cloning studies of human Fc γ Rs. As indicated, each member of this family is an integral membrane glycoprotein (with the exception of the Fc γ RIII expressed in neutrophils), possessing extracellular domains related to a C2-set of immunoglobulin-related domains (5), a single membrane-spanning domain and an intracytoplasmic domain of variable length. These related Fc γ R proteins result from gene duplication and alternative splicing. This general scheme is true for the rodent Fc γ Rs as well. A description of each group, based on these cloning studies, follows.

Fc γ RI

The most distinctive property of Fc γ RI is its relatively high affinity for ligand; it is the only IgG FcR for which the binding of monomeric ligand can be measured directly. Fc γ RI receptor is present on monocytes and macrophages (6, 7); in human neutrophils it is inducible by gamma interferon (IFN- γ) (6) (Table 2). On monocytes and various monocyte lines, expression can be enhanced as much as 20-fold by IFN- γ (8). Human monocytes have a few tens of thousands Fc γ RI (6, 8, 9–12), while murine macrophages have many more (13, 14).

Although no MAbs for muFc γ RI have been reported, several have been

Table 1 Summary of the FcR and associated subunits: genes and transcripts. Characteristics of the genes, transcripts, and predicted proteins for these receptors are based on recent studies of the molecular genetics of this locus. Binding characteristics and ligand affinity are based on previous studies in which differentiation among the various subclasses of Fc Rs was not possible

New nomenclature	Other names	CD, Ly	Mabs	Chromosome	Genes
huFcγRI		CD64	32.3, 22	1q	1
muFcγRI			—	?	1
huFcγRIIA	FcγRIIa	CDw32	2E1, CIKM5 IV.3, 4IH16	1q23	1
B	FcγRIIb, FcγRIIC	CDw32	2E1, CIKM5 IV.3, 4IH16	1q23	1
C	FcγRIIa', FcγRIIB	CDw32	2E1, CIKM5 IV.3, 4IH16	1q23	1
muFcγRII	FcγRIIβ	Ly 17		1q	1
huFcγRIIIAα	III-2	CD16	3G8, B73.1 GRM-1	1q23	1
huFcγRIIIB	III-1	CD16	3G8, B73.1, 1D3 GRANII (NA-1)	1q23	1
muFcγRIIIα	FcγRIIα, FcγRIIA	Ly-17	2.4G2	1q	1
huFcεRIα				1q23	1
muFcεRIα				1q	1
rtFcεRIα			many	?	?
Associated subunits					
muFcεRIβ				19	1
huFcεRIγ				1q23	1
huFcγRIIIAγ					1
muFcεRIγ				1q	1
muFcγRIIIγ		—		1q	1
huFcγRIIIAζ	TCR/CD3ζ	—		1q	1
muFcγRIIIζ	TCR/CD3ζ			1q	1

described that are specific for huFcγRI (Table 1) (15–17). Only one MAb inhibits ligand binding, and this only weakly (17). The others are specific for two different epitopes distinct from the ligand binding site (16). The International Workshop on Differentiation Antigens has recently designated human FcγRI as CD64.

As seen in Tables 1 and 2, cDNA clones for the FcγRI receptors have been isolated and characterized (18–20). In contrast to the two immunoglobulin-like extracellular domains present in all low-affinity FcγRs (Figure 1 and Table 2), FcγRI demonstrates three immunoglobulin-like extra-

Alleles	Transcripts	Affinity for IgG(Ka)	Affinity for IgE(Ka)	Specificity	
				hIgG	mIgG
2	2	10^8 – 10^9		$1 = 3 > 4 \gg 2$	$2a = 3 \gg 1, 2b$
—	1	10^7 – 10^8		$3 > 1 > 4 \gg 2$	$2a, 2b \gg 1, 3$
2 (HR, LR)	2	$< 10^7$		$1 = 3 \gg 2, 4$	$1, 2b \gg 2a, 3$
?	3 (b1, b2, b3)	$< 10^7$		$1 = 3 \gg 2, 4$	$1, 2b \gg 2a, 3$
?	> 1	$< 10^7$		$1 = 3 \gg 2, 4$	$1, 2b \gg 2a, 3$
	2 (b1, b2)	9×10^5		$3 > 1 > 4 \gg 2$	$2b > 2a > 1 \gg 3$
—	1	$< 10^7$		$1, 3 \gg 2, 4$	$3 > 2a > 2b \gg 1$
2 (NA-1, NA-2)	1	$< 10^7$		$1, 3 \gg 2, 4$	$3 > 2a > 2b \gg 2$
—	1	10^6		$3 > 1 > 4 \gg 2$	$2b > 2a > 1 \gg 3$
?	1		10^{10}		
?	1		10^{10}		
?	1		10^{10}		
	2				
	1				
	1				
	1				
	1				
	1				
	1				

cellular domains. Three distinct transcripts have been cloned for huFcγRI, of which two represent polymorphisms and the third demonstrates diversity in the predicted intracytoplasmic domain. However, Southern blot and genomic cloning studies are consistent with a single human FcγRI gene; DNA sequences have not been detected for the third cDNA with its novel cytoplasmic domain. The unusual structure of this cDNA, containing direct and inverted repeats, suggests that a cloning artifact may have occurred (R. N. Pearce, J. V. Ravetch, unpublished). Transcripts of 1.7 kilobases (kb) were detected in RNA prepared from monocytes,

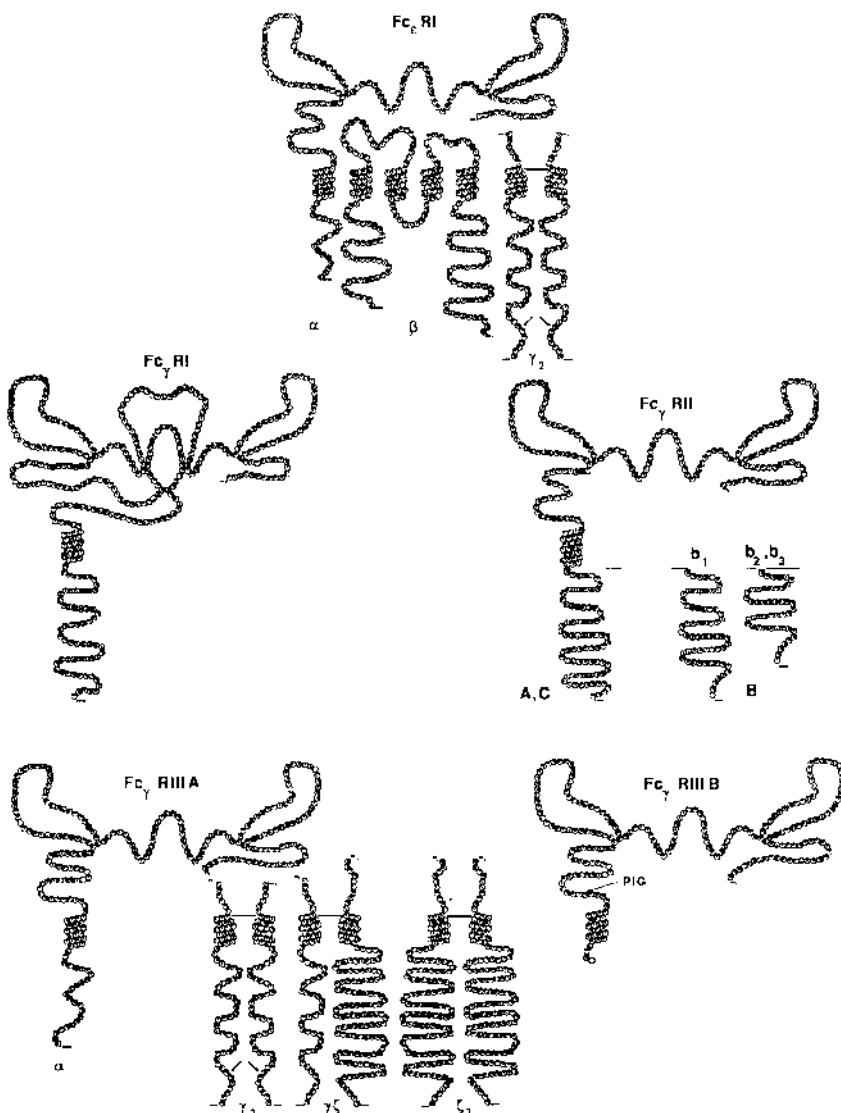


Figure 1 Schematic representation of the proteins encoded by the FcR locus. The protein products predicted from cDNA cloning studies of the rat FcεRI and the human FcγR locus are presented as integral membrane glycoproteins. These proteins are all members of the immunoglobulin super family, as denoted by the Ig-like domains, indicated by the loops, defined by the cysteine residues. The nomenclature is further defined in the text.

Table 2 Fcγ locus: predicted proteins

	Glycosylation sites (Number)	Associated subunits	Molecular forms	Number of amino acid residues				IC	Molecular weight	
				Total	LP	EC	TM		Apparent	Protein backbone ¹
huFcγRI	6		single chain	374	15	273	21	63	72	40
muFcγRI	5	—	single chain	404	24	273	23	84	67	42
huFcγRIIA	2	—	single chain	325	35	186	28	76	40	31
huFcγRIIB b1	3		single chain	310	44	180	23	63	40	29
b2	3		single chain	291	44	180	23	49	40	27
huFcγRIIC	3		single chain	321	42	178	23	78	40	31
muFcγRII b1	4		single chain	329	29	180	26	94	40–60	33
b2	4		single chain	282	29	180	26	47	40–60	29
huFcγRIIIAα	5	+	$\alpha\gamma_2, \alpha\gamma_5, \alpha\zeta_2$	254	17	191	21	25	50–80	33
huFcγRIIIB	6	—	GPI anchored	233	17	191	21	4	50–80	29
muFcγRIIIα	4	+	$\alpha\gamma_2, \alpha\gamma_5$	261	30	184	21	26	40–60	33
huFcεRIα	6	+	$\alpha\beta\gamma_2$	260	28	180	21	31	45–65	26.4
muFcεRIα	6	+	$\alpha\beta\gamma_2$	250	23	181	21	25	45–65	25.8
rtFcεRIα	7	+	$\alpha\beta\gamma_2$	245	23	181	21	20	45–65	25.2
Associated subunits										
muFcεRIβ	2	+	$\alpha\beta\gamma_2$	235 (243)	—	EC1	TM1	IC N _{term}	32 (32)	25.9 (27)
rtFcεRIβ	2	+	$\alpha\beta\gamma_2$			17 (17)	21 (21)	51 (59)		
						EC2	TM2	IC loop		
						29 (29)	21 (21)	11 (11)		
							TM3	IC C _{term}		
							21 (21)	43 (43)		
							TM4			
							21 (21)			
huFcεRIγ	0	+	$\alpha\beta\gamma_2$	86	18	5	21	42	7–9	7.8
huFcγRIIIAγ	0	+	$\alpha\gamma_2, \alpha\gamma_5$	86	18	5	21	42	7–9	7.8
muFcεRIγ	0	+	$\alpha\beta\gamma_2$	86	18	5	21	42	7–9	7.8
muFcRIIIγ	0	+	$\alpha\gamma_2, \alpha\gamma_5$	86	18	5	21	42	7–9	7.8
huFcγRIIIAζ	0	+	$\alpha\zeta_2, \alpha\gamma_5$	163	21	9	21	112	16	16.1
muFcγRIIIζ	0	+	$\alpha\gamma_5$	164	21	9	21	112	16	16.2
rtFcεRIγ	0	+	$\alpha\beta\gamma_2$	86	18	5	21	42	7–9	7.8

¹ Processed

placenta, and U937 cells (where a second transcript of 1.6 kb was detected); these transcripts were inducible by IFN- γ (Table 3). Transfection of these clones in COS cells resulted in surface molecules that had a relative molecular size of 70 kd, which bound IgG with an affinity and specificity similar to those determined from in vivo studies, and which reacted with Fc γ RI-specific MAbs. The first two extracellular immunoglobulin-like domains were homologous to the extracellular domains of Fc γ RII and III, while the third showed little similarity. We suggest the presence of this third domain may account for the high-affinity binding of this receptor. cDNA clones have recently been identified for a murine Fc γ RI (20). The predicted amino acid sequence reveals 65–75% identity to huFc γ RI in the three extracellular domains and the transmembrane domain. However, the cytoplasmic domain is 22 amino acids longer (Table 2) (84 vs 63) and is only 25% identical to huFc γ RI.

Table 3 Expression of Fc receptors in hematopoietic cells. (Data are based on expression of RNA for the indicated genes, using probes that differentiate among the highly homologous members of a particular group.)

	M ϕ	Mono	Neut	FcR family				
				NK	B	T	Mast	Baso
huFc γ RI	+	+	+, i			..	-	-
muFc γ RI	+	?	?	-	-	-	..	-
huFc γ RIIA	+	+	+	-	-	-	?	?
huFc γ RIIB	+	+	-	-	+		?	?
huFc γ RIIC	+	+	+	-	-	-/? ^a	?	?
muFc γ RII	+	+	+		+	+	+	?
huFc γ RIIIA α	+	+, i		+		+ ^b	?	?
huFc γ RIIIB		-	+	-	-	-	?	?
muFc γ RIII α	+, i	?	+	+	-	-	+	?
huFc ϵ RI α	-	-	-	-	-	-	+	+
muFc ϵ RI α	-	-	-	-	-	-	+	+
Associated subunits								
muFc ϵ RI β		-	-				+	+
{ huFc ϵ RI γ huFc γ RIIIA γ }	+	+	+/- ^c	+	-	+	+	+
{ muFc ϵ RI γ muFc γ RIII γ }	+	?	?	+	-	+	+	+
huFc γ RIIIA ζ	-		-	+	-	+	-	-
muFc γ RIII ζ	-	-	-	+	-	+	-	-

^a Neg in T-cell lines (Molt-4, Jurkat, Fro-2), peripheral not tested.
^b Only on T γ subset and CD16/CD3+ lymphocytosis.
^c Neg in peripheral PMN, pos in HL-60 treated with DMSO.
i = inducible.

FcγRII

The FcγRII molecules are remarkably widespread. They are found on virtually every FcγR-bearing cell with the exception of the NK cell (Table 3). Their cell numbers vary greatly, from 10^3 on platelets to more than 10^5 on murine macrophages (21, 22). Unlike FcγRI, the affinity of FcγRII for ligand is so low that binding of monomeric ligand either cannot be measured directly or is on the border of detectability; the equilibrium affinity constants (K_a) are less than 10^7 M^{-1} (22–25) (Table 1). Nevertheless, the presence and function of FcγRII are readily demonstrated by the binding of immune complexes.

Several MABs have been raised to huFcγRII and assigned the designation CDw32. All of the well-characterized MABs block ligand binding, unlike most of those specific for huFcγRI. Two of the MABs, IV3 (7, 26) and KuFc79 (27), appear to define cell-specific epitopes. MAB 41H.16 recognizes an epitope common to the responder allele (HR) and to FcγRII in general; this epitope is lost in the nonresponder (LR) receptor. The FcγRII of murine cells is recognized by MAB 2.4G2 (28).

Much is known regarding the structure, expression, and distribution of FcγRII genes and transcripts (Tables 1, 2, 3). Molecular clones have been described for both murine (29–31) and human (32–36) molecules, revealing an intriguing pattern of evolutionary relationships. The prototype molecule of this group is the murine FcγRII gene and its transcripts. When expressed by transfected fibroblasts this gene encodes a low affinity receptor, binding mIgG1, mIgG2b, and mIgG2a (37). Two distinct cDNAs have been isolated and characterized; these are referred to as b1 and b2. These molecules are derived by alternative splicing of cytoplasmic encoding exons (Figure 2) (38). The corresponding proteins differ by a 47-amino-acid insertion in the cytoplasmic domain (b1). The b1 form is preferentially expressed in lymphocytes and the b2 form in macrophages.

In contrast to this relatively straightforward murine system, FcγRII in humans is a complex genetic locus, encoded by a minimum of three genes (see below) (38) which results in six distinct transcripts (35) (Table 1 and Figures 1, 2). These genes and their transcripts are evolutionarily related to the murine FcγRII gene, with several interesting twists. Three distinct transcripts are derived from the IIB gene by alternative splicing of cytoplasmic exons (b1 and b2) (as was seen in the murine FcγII transcripts), with an additional form derived from alternative splicing of the exons encoding the signal sequence (b3) (35, 38). Northern (RNA) blot analyses of IIB transcripts reveal that they are distributed in monocytes, macrophages, lymphocytes, and various pluripotential cell lines (Table 3). Expression has not been detected in NK cells, neutrophils, or T-cell lines.

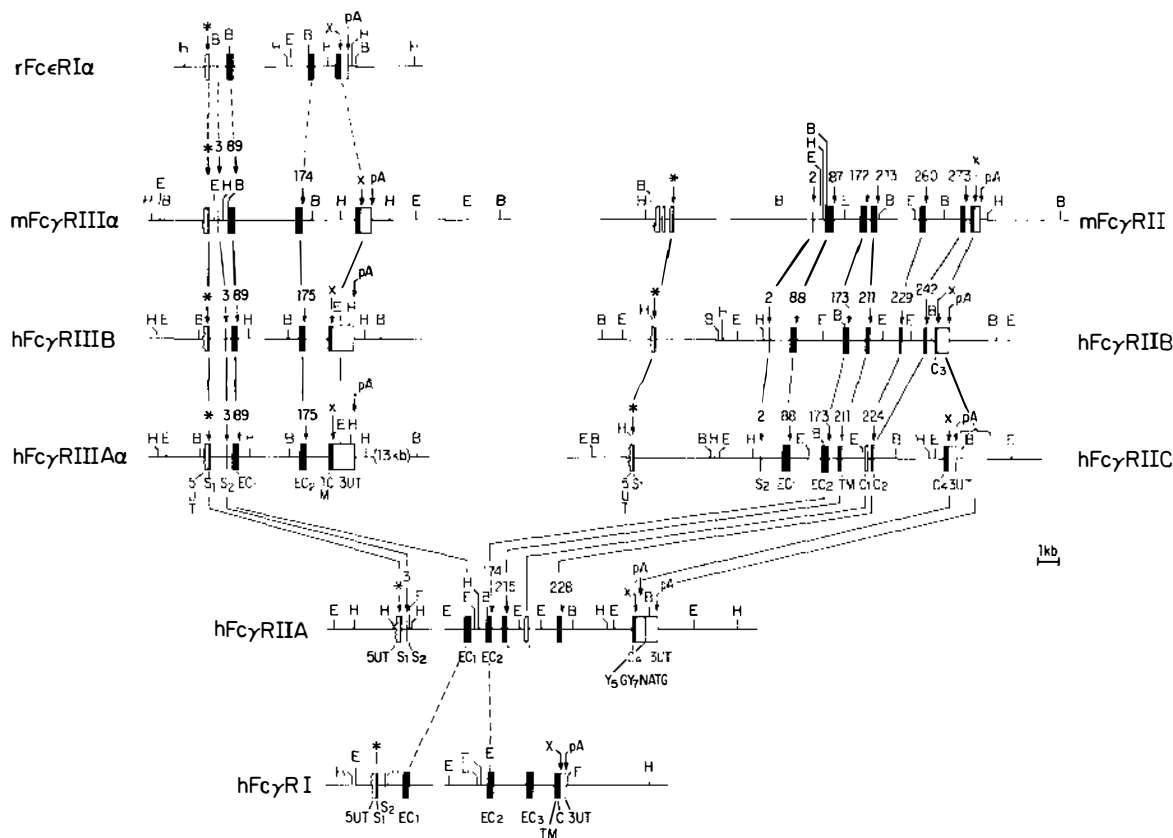


Figure 2 Genomic organization and evolutionary relationships among FcR genes. The intron-exon organization of each gene is indicated, with exons denoted by rectangles and introns by solid lines. Filled rectangles represent translated regions; open rectangles indicate transcribed but untranslated sequences (with the exception of the C_1 exon on IIA,C). Connecting lines indicate conservation of sequences among exons of a particular gene family. Abbreviations for exons are: S, signal; EC, extracellular; TM, transmembrane; C, cytoplasmic; UTR, untranslated. *

After transfection of IIB cDNAs into fibroblasts all three spliced forms expressed cell surface molecules which bound IgG with low affinity, and which were recognized by the MAbs of the CDw32 cluster. In addition to these transcripts, three other transcripts have been identified in the Fc γ RII group of molecules (35, 36). These transcripts, known as IIA and IIC, are derived from two distinct genes. Extensive sequence homology exists between IIB and these Fc γ RII molecules in their extracellular domains, transmembrane domain, and 3' untranslated sequences; IIB sequences differ from IIA only in the signal sequence and in a part of the cytoplasmic domain while IIB differ from IIC only in the cytoplasmic domain. IIA gives rise to two transcripts of 1.8 and 2.5 kb as a result of alternative polyadenylation; IIC is encoded by a distinct gene, yielding a transcript of 1.8 kb. The feature that distinguishes IIA and IIC is their signal sequences. The IIA signal sequence is homologous to the murine Fc γ RIII sequence, while the IIC signal is homologous to the murine Fc γ RII signal. Otherwise, the transcripts are nearly identical. These genes generate different transcripts, however, which can be distinguished by the polymerase chain reaction (PCR). IIA and IIC are expressed in monocytes, neutrophils, and some pluripotential cells like HL-60 and K562. They are not expressed in lymphocytes and NK cells. Both IIA and IIC give rise to the same final protein product (albeit with several amino acid substitutions) and have not been distinguished by MAbs.

HuFc γ RIIB molecules are preferentially expressed in lymphocytes, whereas IIA and IIC are preferentially expressed in neutrophils; monocytes express both classes (Table 3). This selectivity of expression of molecules having nearly identical extracellular and transmembrane domains coupled to divergent cytoplasmic domains is a common theme among Fc γ Rs.

Transfected IIA binds IgG with low affinity and is recognized by MAbs of the CDw32 cluster. By such analysis, MAbs that distinguish rigorously among IIA, IIB, and IIC have not been seen, although some quantitative differences are apparent, indicative of differences in antibody affinity or titer. These differences may account for the apparent specificity of certain CDw32 MAbs for lymphocytes, which, by Northern blot analysis, express IIB preferentially. Alleles of IIA have been described, based on their ability to bind (high responder, HR) or not bind (low responder, LR) mouse IgG. A single amino acid substitution in Fc γ RIIA is responsible for the allelism (R¹³¹-H¹³¹) (39).

Fc γ RIII

Mu Fc γ RIII and the huFc γ RIII genes are now recognized as homologous molecules by virtue of their sequence homology, genomic organization, cellular distribution, and shared function. Murine Fc γ RIII was first iden-

tified in murine macrophage cell lines (29) and was classified as an Fc γ RII molecule because it reacted with MAb 2.4G2 (37). A single transcript of 1.6 kb has been identified for murine Fc γ RIII. Murine Fc γ RIII shows 95% sequence conservation in its extracellular domains compared to Fc γ RII, but its transmembrane and cytoplasmic domains are distinctly unrelated. Transfection of Fc γ RIII resulted in the appearance of a low-affinity receptor which had the binding affinity and specificity identical to that of Fc γ RII (37), but the efficiency of expression of murine Fc γ RIII was only 1 to 2% that of Fc γ RII transfected into COS or L cells. Fifty- to 100-fold stimulation of surface expression was seen by the coexpression of a second molecule, the γ chain of Fc ϵ RI (40, 41). The γ -chain transcripts are found in NK cells and macrophages (Table 3) that do not express Fc ϵ RI but do express muFc γ RIII, suggesting a physiological role for this molecule in murine Fc γ RIII expression, function, or both. The distribution of Fc γ RIII is restricted; it is seen only in macrophages and macrophage cell lines, NK cells, myeloid precursor cells, and a neutrophil cell line (29, 37; Ravetch, unpublished data: Table 3). The suggestion that murine Fc γ RIII mediated a different function than Fc γ RII followed the recognition that it was the only Fc γ R on NK cells, where it mediates antibody-dependent cellular cytotoxicity (ADCC) (42). Fc γ RIII expression on macrophages is modulated by IFN- γ , where its expression parallels the cells' acquisition of phagocytic capacity (37). In thioglycollate-elicited macrophages, Fc γ RIII is preferentially expressed as compared to Fc γ RII, suggesting that immune complexes may be targetted to Fc γ RIII to trigger macrophage effector phenotypes.

These characteristics of murine Fc γ RIII are also seen in the human system, again with somewhat greater complexity. Two distinct forms of huFc γ RIII (Fc γ RIIIA and Fc γ RIIIB) have been defined by studies employing leukocytes from individuals with paroxysmal nocturnal hemoglobinuria and using phosphatidylinositol-specific phospholipase C (43–45). The form found on neutrophils, IIB (46), is anchored to the outer leaflet of the plasma membrane by a glycosyl-phosphatidylinositol (GPI) moiety, whereas the form of the receptor expressed on NK cells, macrophages (46; B. Perussia, J. V. Ravetch, *E. J. Immunol.*), and cultured monocytes, IIIA (43), is a conventional transmembrane protein with a distinct cytoplasmic domain. Among the several MAbs available against huFc γ RIII (designated CD16) (47–54) MAb 1D3 appears to discern this heterogeneity. In addition MAb B73.1 appears to recognize an epitope that is somewhat specific for the NK cell Fc γ RIII (55).

An alloantigen (NA) recognized by an autoantibody present in the serum of patients with several forms of autoimmune neutropenia is present on huFc γ RIIIB (53). MAbs are now available that recognize these two

allotypic forms of the receptor, which can also be distinguished by their mobility on sizing gels (56, 57). A single amino acid exchange in EC1 of IIIB accounts for this allelism. IIIB is expressed exclusively in neutrophils, while IIIA is expressed in NK cells and macrophages (46; Table 3). The IIIB transcript generates a GPI-linked molecule on neutrophils (43–46, 58); the IIIA transcript is translated into an integral membrane glycoprotein (46, 59), as is its murine counterpart. A single amino acid exchange determines the alternative membrane anchors of IIIB and IIIA. Ser-203, as found in IIIB, results in a GPI-linked protein. Phe-203, as found in IIIA, prevents the formation of GPI anchor. This amino acid difference occurs at the site of GPI linkage (41, 60, 61). IIIA encodes a 25-amino-acid cytoplasmic domain, homologous to murine Fc γ RIII, as a result of a T-to-C transition in the termination codon TGA, found in the IIIB gene. By virtue of the minimal nucleotide substitutions relative to IIIB, IIIA results in a transmembrane anchored protein with a cytoplasmic domain, clearly homologous to murine Fc γ RIII. Profound functional consequences result from these subtle changes in the Fc γ RIII genes, with IIIA expressed on NK cells and macrophages which are capable of mediating ADCC and phagocytosis (62–64), while IIIB is not. It has been postulated, that GPI-anchored Fc γ R serves as a trap for immune complexes in the peripheral circulation which do not activate neutrophils (43, 44). On the other hand IIIB may act synergistically with IIA, expressed on neutrophils, to mediate ADCC and phagocytosis, suggesting that a threshold concentration has to be achieved before IIIB can interact with and trigger via IIA. Like its murine counterpart, efficient expression of IIIA requires coexpression of Fc ϵ RI γ chain or a homologous protein, the ζ chain of the CD3/TcR complex (41, 65). A similar requirement was not found for the GPI-linked molecule IIIB (41, 60).

STRUCTURE-FUNCTION RELATIONSHIP OF Fc γ Rs

The assignment of specific functions to this wealth of structural diversity for Fc γ Rs will undoubtedly be the focus of research for the next several years. At present, some preliminary correlations can be discerned, based primarily on the restricted cellular expression for some of these molecules. In addition, analysis of these molecules expressed in heterologous cells (COS, CHO, L cells) offers some clues about the different biological properties of these related molecules, albeit in an artificial context. Accurate assignment of functions to specific structures will require the re-introduction of both native and mutated versions of these molecules into cells capable of Fc γ R-mediated responses. Such studies are in progress in several laboratories. Some general observations emerge from the studies

performed over the past decade: Fc γ R_s appear to have overlapping biological activities such that each group may mediate several effector functions, and cross-linking of these receptors by ligand appears to be necessary to trigger these functions.

In both mice and humans, Fc γ R_{II} appear to be the most widely distributed; they are found on most hematopoietic cells. The selectivity of alternative splicing of the murine Fc γ R_{II} gene, with b2 exclusively expressed in myeloid cells and b1 expressed in lymphocytes and early myeloid cells, suggests functional roles for the alternative cytoplasmic domains of this molecule. Recent studies in transfected fibroblasts (66) have shown that these two molecules process immune complexes differently, with b2 mediating ligand internalization and delivery to lysosomes while b1 fails to do so. The 47-amino-acid insertion in the cytoplasmic domain of b1 has also been shown to be a site of serine phosphorylation (67). However, similar regulation of alternative splicing of the human IIB gene has not been observed (35). In addition, the homologous, alternatively spliced C1 exon of human IIB is truncated so that the phosphorylation sites found in the mouse b1 protein are not conserved. Thus, the role of Fc γ R_{II}b1 phosphorylation in B-cell activation remains to be determined.

Recent studies (K. A. Joiner et al 1990, Science 249, 641) using CHO cells transfected with murine Fc γ R_{II} (both b1 and b2 spliced forms) have suggested that for particular IgG-coated ligands this Fc γ R can enhance internalization. The protozoan parasite *Toxoplasma gondii* normally infects mammalian cells; heat-killed parasites cannot. Coating these heat-killed parasites with IgG resulted in enhanced internalization into Fc γ R_{II}B transfectants and was dependent upon an intact cytoplasmic tail. Other immune complexes such as IgG coated erythrocytes bind efficiently but are not internalized by these transfectants, in contrast to what is observed on macrophages. Thus, the binding of the parasite to other surface molecules appears to play a role in facilitating Fc γ R mediated entry into these cells. Whether this represents a specialized case or is indicative of a general feature of Fc γ R_{II}B is still to be clarified.

IIA and IIC are the only Fc γ R_{II} transcripts expressed in human neutrophils, with IIB expression notably absent (35). IIA is not expressed in any human B- or T- lymphocyte cell lines examined to date. These data suggest that IIA and IIC, which encode nearly identical proteins (>95% identity), are restricted to effector cells like monocytes, macrophages, and neutrophils (though not NK cells) and are capable of mediating Fc γ R_{II}-triggered effector responses like phagocytosis and ADCC. IIB is more widely distributed and is the only Fc γ R_{II} expressed on lymphocytes. It appears that IIA and IIC mediate overlapping "effector" functions, whereas IIB is more related to lymphocyte properties.

The Fc γ RIII α and Fc γ RIIIA α molecules offer the only clear association of Fc-mediated structure-function relationship. NK cells are unique among effector cells in expressing only a single Fc γ R molecule, III α in mouse cells (42) and its homolog IIIA α in human cells (46). The conservation of amino acid sequence in the transmembrane and to a lesser extent in the cytoplasmic domains of these receptors suggests that these domains are essential for functions such as ADCC. Although the macrophage expresses all three groups of Fc γ R molecules, it regulates the expression of III α and IIIA α so that the activated cell preferentially directs immune complexes to this low-affinity receptor. Fc γ RIII molecules associate with γ and ζ chains, as discussed below. Different combinations of homo and hetero dimers of the γ and ζ chains have been observed in human NK cells (68), offering the possibility of different signalling pathways through these receptors, as has been suggested for TCR/CD3 (69).

MECHANISM OF ACTION OF Fc γ Rs

While it is clear that Fc γ Rs are signal transducing molecules, few of the experiments performed can be clearly interpreted, in light of the considerable structural heterogeneity of these molecules on most cell types. Thus, stimulation of murine macrophages with immune complexes or anti-Fc γ R mAbs induces an increase in $[Ca^{2+}]_i$ (70), although whether Fc γ R II or Fc γ R III is mediating this response is not clear. Similarly, human monocytes (71) and neutrophils (72) can be stimulated with an anti-Fc γ R II mAb and can induce similar increases in intracellular calcium. Cross-linking of at least two receptor molecules is necessary, and increased rises in intracellular calcium occur upon further cross-linking. Once again, the mAbs used in these studies do not distinguish among the multiple Fc γ R II molecules, so that simple interpretation of these results is difficult.

Stimulation of Fc γ RIII on NK cells with ligands results in a rapid rise in $[Ca^{2+}]_i$ and the hydrolysis of membrane phosphoinositides, resulting in the production of both inositol 1,4,5 triphosphate (IP₃) and IP₄ (73). Further cross-linking of the receptor augments these responses. Part of the initial $[Ca^{2+}]_i$ rise depends on mobilization from intracellular stores, but sustained $[Ca^{2+}]_i$ levels require the influx of extracellular calcium. NK-cell activation, mediated by Fc γ RIII cross-linking, also results in transcriptional activation of specific cytokines like IFN- γ and TNF (74). This activation is dependent upon extracellular calcium influx.

In some studies GPI-anchored Fc γ RIIIB was shown to bind ligand but not to induce phagocytosis or ADCC (75, 76). Consistent with those experiments are the observations that a rise in $[Ca^{2+}]_i$ and hydrolysis of phosphoinositides are not induced upon cross-linking of Fc γ RIIIB (P.

Perussia, unpublished). Conflicting results have been reported (77, 78), but these suffer from the ambiguity that the ligands used to trigger Fc γ RIII may also be capable of triggering Fc γ RII molecules.

Resolution of the different mechanisms of Fc γ R signalling will require the introduction of the individual ligand binding subunits and associated chains into cells that are capable of Fc γ R function but deficient in specific subunits. Such cell types are becoming available and will clarify these pathways.

FC RECEPTOR FOR IMMUNOGLOBULIN E (Fc ϵ RI)

The high affinity receptor for IgE (Fc ϵ RI) is the surface component that initiates allergic reactions (see also reviews 79–81). Unlike the Fc γ receptors described above, Fc ϵ RI is only present on the surface of mast cells and basophils. Activation of Fc ϵ RI induces several immediate cellular events: cellular degranulation and the release of granular contents such as histamine, the biosynthesis and the secretion of arachidonic acid metabolites such as leukotrienes (reviewed in 82). In addition, the activation of Fc ϵ RI results in the synthesis and in some cases the secretion of many cytokines (83–85) including GM-CSF, IL-1 α , IL-3, IL-4, IL-5, IL-6, TNF- α , IFN- γ , and also four members of the MIP-1 gene family (TCA3, JE, MIP-1 α and MIP-1 β).

Cell activation through Fc ϵ RI requires two independent binding events. The soluble IgE secreted by B cells must first bind to the cell surface receptor. However, this binding of ligand is not sufficient to activate the cell. Interaction of receptor-bound IgE with a multivalent antigen is necessary to initiate cell activation.

A Multimeric Complex $\alpha\beta\gamma_2$

During the initial attempts to purify the receptor by affinity chromatography, a single polypeptide was identified (86–88). This glycoprotein—the α subunit—has an apparent mol wt of 50–60 kd on sodium dodecyl sulfate polyacrylamide gels. Other studies indicated that the receptor might consist of more than a single polypeptide. In particular, experiments with chemical cross-linking reagents (89) suggested an association of α with other subunits. Later on, the IgE-binding α chain was found to be associated with one β chain and two disulfide-linked γ chains (90). The initial failure to detect the associated subunits was due to the easy dissociability of the tetrameric complex in mild detergent (91, 92), the detergent micelles disrupting the noncovalent interactions between the subunits. However, these interactions are protected in solutions of sub-

micellar detergent or when detergent micelles are mixed with specific phospholipids (92, 93, see also review 94). Finding the conditions to keep the complex together was instrumental in the purification of the individual subunits. Microsequencing of purified tryptic peptides allowed the cloning of complementary DNAs for each of the subunits and the isolation of the corresponding genes.

Transcripts and Polypeptide Structure of the α , β , and γ Subunits

THE α SUBUNIT Complementary DNAs for the α subunit have been isolated from three different species (95–99). As expected, the corresponding transcripts were found exclusively in cells of mast cell or basophil lineage. One major species of mRNA was found both in rodents (1.3 kb) and in humans (1.1 kb). Three additional transcripts were identified from cDNA cloning. However, the genomic structure of the α gene (100) indicates that these various transcripts are most likely generated from aberrant splicing or unspliced precursors. The deduced amino-acid sequence of rat, mouse, and human α comprises 245, 250, and 260 residues, respectively. The difference of length among the species is mainly due to a difference in the length of the cytoplasmic tails: 20, 25, and 31 residues for the rat, mouse, and human α , respectively. A hydropathicity plot of the sequence from each of the three species shows a characteristic N-terminal signal peptide and a single transmembrane domain. The extracellular domain of about 180 residues contains two immunoglobulin-related domains of 40 and 42 residues, respectively. These domains each contain a pair of cysteines which most likely generate disulfide bridges. The extracellular portion of α contains seven (rat) or six (mouse and human) potential sites for N-linked glycosylation. The position of only one of these sites is conserved among the three different species. Although conservation of the DNA sequence between the rodent and human α chains is substantial (68% homology), only 38% identical amino-acid residues exist in the consensus sequence for the three species. The level of conservation differs among the different domains. There is only 16% identity in the cytoplasmic tail, while 62% of the transmembrane residues are identical, and nine are consecutive. Interestingly, eight of these nine residues are also conserved in the transmembrane domain of the homologous mouse and human Fc γ RIII. These residues may be involved in the interaction with the γ chains associated to these receptors (see below). In addition, the two immunoglobulin-related domains of the extracellular domain of Fc γ RIII are homologous both in size and sequence to the corresponding domains in α . Among a total of 95 amino acids of α found to be identical in three species (see above), 61%

of these are also found in both human and mouse Fc γ RIII. Therefore, only 34 residues in α seem specific for IgE.

THE β SUBUNIT Complementary DNAs for rat and mouse β have been described (98, 101). The cloning of human β has not yet been reported, but clones have now been isolated and are currently being characterized (H. Kuster, J.-P. Kinet, unpublished). The open reading frame in the rat sequence is slightly longer than that in the mouse sequence—729 and 705 bp, respectively. This is due to a 24-bp in-frame insertion in the rat sequence. Two major species of mRNA (2.7 kb and 1.75 kb) were observed in rodent mast cells but neither in any other cell lines tested. These two transcripts are the results of alternate polyadenylation. Other cDNA clones with an early termination are the likely result of aberrant splicing. The deduced amino-acid sequence of rat and mouse β comprises 243 and 235 residues, respectively, with no apparent leader peptide. The level of homology between rat and mouse β is equivalent, whether DNA sequences (87%) or amino-acid sequences (83% identical residues) are compared. Preliminary results on the human β indicate that about 70% of the amino-acid residues are identical between human and rodent. A hydropathicity plot and other studies using monoclonal antibodies (101) suggest that β crosses the plasma membrane four times and has both amino and carboxy termini protruding into the cytoplasm (Table 3).

THE γ SUBUNIT The cDNA sequences of rat, mouse, and human γ have now been reported (98, 102, 103). A single corresponding transcript (0.85 kb) has been detected in the three species. Unlike the case for α and β , the transcripts for γ have been identified in many hematopoietic cell lines (see below). The γ subunit from rat, mouse, and human receptors is highly conserved, and the consensus sequence for the three species contains 86% identical amino-acid residues. A hydropathicity plot of the sequence shows a typical leader peptide and a single transmembrane domain. The N-processing should cleave 18 residues to yield a membrane-attached peptide of 68 residues with a molecular weight of about 7.8 KDa. This is in good agreement with previous estimates using SDS-Urea gels for γ purified from the surface (104). Because the γ subunit is not seen labeled after biosynthetic incorporation of [3H]histidine when purified from the plasma membrane, we had initially speculated that a posttranslational processing of the C-terminal end of γ could remove the unique histidine residue (102). However, by using an antipeptide antibody directed against the histidine-containing C-terminal peptide of γ , we now have preliminary evidence that this processing is not occurring (O. Letourneur and J.-P. Kinet, unpub-

lished). There are two cysteine residues in the γ sequence located at each end of the plasma membrane. Recent data using mutated γ chains (105) indicate that dimerization of γ chains occurs via the N-terminal cysteine only. This cysteine is analogous to the single cysteine in the ζ chain of the T-cell receptor (see below). The topology of γ chains is based on two types of studies. Surface iodination of intact cells does not label γ chains, but γ can be iodinated from inverted vesicles (106). Furthermore, in vivo studies indicate that threonine residues of γ are phosphorylated (107). Since tyrosine and threonine residues are only found in the presumptive cytoplasmic tail, it is likely that the short segment of five residues preceding the transmembrane domain is extracellular.

Ontogeny, Biosynthesis, Assembly, and Catabolism

Culture of bone marrow cells in the presence of interleukin-3 indicates that receptor expression occurs very early during the differentiation of mouse mast cells and human basophils (108). Even undifferentiated cells already express Fc ϵ RI on their surface. These cells most likely represent committed progenitors. Whether these nongranulated cells can be stimulated through Fc ϵ RI to synthesize and secrete cytokines is not yet known.

If the relative abundance of mRNA for the subunits correlates with the amount synthesized, then the subunits are probably not synthesized in equal amounts. As has been observed for other multimeric complexes, it is possible that the amount of one of the subunits is the limiting factor for receptor assembly and consequently for surface expression (109). At present how the tetrameric complex assembles is not clear. However, the surface receptor apparently recycles and is degraded as a unit (110). Thus, the upregulation of cell surface receptors upon binding of monomeric IgE may possibly result from decreased degradation of the receptor because it is protected by the bound IgE from enzymatic degradation during receptor recycling (110, 111).

Expression of Fc ϵ RI by Gene Transfer

In the first studies with the rat α gene, no expression of IgE binding was detected on the surface of transiently transfected Cos-7 cells (95, 97). In fact, surface expression of rat, mouse, or human α occurs, but with an efficiency so low detection is difficult (98). The requirements for efficient receptor expression are different for the rodent and the human receptor. Simultaneous cotransfection of the three genes for α , β , and γ is necessary to induce surface expression of the rodent receptor (102), while cotransfection of human α and γ is sufficient, as if β was not necessary. The same is true whether human α is transfected with rat, mouse, or human γ (98,

103, 112). These results leave open the possibility that human $\alpha(\gamma)_n$ receptor complexes occur in vivo. With the availability of a human β probe, it should now be possible to investigate this interesting question.

Interactions Between IgE and Fc ϵ RI

The binding of IgE to Fc ϵ RI is relatively specific. Other isotypes do not compete with IgE, but the species specificity is not complete. For example, human receptors bind human but also rat and mouse IgE (113). The receptors are univalent (114) and bind monomeric IgE with an affinity of about 10^{10} M^{-1} (112, 115, 116). The stoichiometry of one α molecule per bound IgE was established by several kinds of studies and is consistent with the analysis of soluble IgE-receptor complexes by electron microscopy, which did not detect more than one IgE bound receptor (M. Phillips, V. Shumacker, H. Metzger, J.-P. Kinet, unpublished).

Recently a recombinant unglycosylated peptide was shown to inhibit human IgE-mediated activation (117). This 76-amino-acid peptide corresponds to a region (residues 301–376) at the junction of C ϵ 2 and C ϵ 3. The same study suggests that only one of the ϵ chains is involved in binding to the receptor. Now that cells expressing the human receptor are available (112), it should be possible to use this peptide in more complete binding studies in order to test whether this linear region of the ϵ chain is necessary and sufficient for IgE binding.

Studies with engineered mutants of the α chain show that the extracellular portion of the α chain is sufficient to promote high affinity binding to IgE (U. Blank, C. Ra, J.-P. Kinet, *J.B.C.* In press). These studies also indicate that β and γ subunits are not required or involved in the binding reaction. Furthermore, it appears that at least two regions of the extracellular domain of α are involved in forming the pocket responsible for the binding.

MECHANISMS OF ACTION OF Fc ϵ RI

Role of Aggregation

Aggregation of receptors has long been recognized as important and has been analyzed extensively in previous reviews (79). Anti- α antibodies, but not their Fab fragments, are able to trigger exocytosis (118, 119). Similarly, artificially cross-linked IgE but not monomers stimulate cell release. The minimum size of the aggregate appears to be a dimer (120, 121), and a few thousands of receptor aggregates are sufficient (122, 123). However, studies with fluorescent probes suggest that small receptor aggregates tend to form larger patches spontaneously (124). Therefore, it is unclear what the minimum size of an "active" aggregate is. The question of whether physical

contact between receptor molecules is necessary for signaling has also been investigated (125). The distance between two epitopes of a rigid bivalent hapten was varied in order to probe the distance between receptors aggregated via IgEs specific for this hapten. When the distance between the two epitopes of the hapten was apparently large enough to prevent physical contact between aggregated receptors, the bivalent haptens were still capable of triggering. Therefore, contact between adjacent receptors is apparently not necessary for cell activation. It has also been suggested that constraints of orientation determine the magnitude of secretory signals (126). If so, this could explain why anti- β antibodies fail to trigger cell activation (127). The old observation that exocytosis and signaling induced by a multivalent hapten can be interrupted by an excess of monovalent hapten has been given a new interpretation (128). The role of monovalent hapten apparently is not so much to disaggregate previously aggregated receptors but to prevent further receptor aggregation. Since signaling is halted after addition of monovalent hapten, although aggregated receptors remain on the surface it is proposed that these receptors must have been desensitized. Therefore, the analysis of possible structural changes of "active" receptors such as phosphorylation should be performed very early after stimulation.

Role of Phosphorylation

Two issues have to be considered: the phosphorylation of the receptor itself and the receptor-induced phosphorylation of adjacent molecules. In view of the previous paragraph, it is possible that past studies on the phosphorylation of the receptor did not look at "active" receptors, since the analysis was done after 15 min of stimulation (107). In those studies, the level of phosphorylation of β appears to increase upon IgE-induced cell stimulation while γ become less phosphorylated. The phosphorylated amino-acid residues of β and γ isolated from unstimulated cells are serine and threonine, respectively (107, 129). The identification of the amino acid residues modified after cell stimulation has never been reported. What these changes might mean and whether new information would be gained from reanalysis of "active receptor complexes" is unclear.

Aggregation of receptor induces phosphorylation of specific substrates via at least one tyrosine kinase (130). Furthermore antibodies against the surface-expressed tyrosine phosphatase CD45 inhibit IgE receptor-mediated histamine release from human basophils (131). This suggests that tyrosine phosphorylation plays a role in linking receptor triggering to exocytosis. Although the receptor itself is not a tyrosine kinase, it may be closely associated with such a kinase. This could explain why tyrosine kinase activity copurifies with IgE receptor complexes (129).

Calcium, GTP Binding Proteins, Phospholipid Hydrolysis and Protein Kinase C

Ample evidence now exists that aggregation of receptors induces a rise of cytoplasmic calcium due to a mobilization of intracellular stores which is itself followed by an influx of extracellular calcium (132–134). How these two events are related and their relative importance are still unclear. The mechanism of the initial release most likely involves a product of the phosphoinositides (PI), inositol 1,4,5 triphosphate (134, 135). This second messenger induces the release of calcium from the endoplasmic reticulum but also appears to stimulate a calcium influx associated with whole-cell current of 1–2 pA or less, with no channel activity detectable in whole-cell patch clamp recording (134). Cell activation results also in the opening of large conductance (50 pS) cation channels. The latter represent a second pathway for Ca^{2+} influx which can be activated by $\text{GTP}\gamma\text{S}$ and inhibited by $\text{GDP}\beta\text{S}$ (134). These results are consistent with other studies in which calcium influx was shown to be under the control of a cholera toxin-sensitive GTP-binding protein (136, 137) and to occur via a pathway independent from PI hydrolysis. That calcium enters the cell under physiologic conditions of triggering is certain, but studies with dialysed mast cells indicate that Ca^{2+} is neither necessary nor sufficient for exocytosis (138). In addition, under certain conditions permeabilized cells can degranulate in the total absence of calcium (139). It is likely that under physiological conditions calcium acts synergistically with other second messenger systems to ensure fast and efficient secretion (140). Some investigators claim to have purified specific channels which open when co-aggregated with the receptor (141). However, these results have been controversial and criticized in the past (79). It is likely that nothing short of the cloning and the genetic reconstitution of these molecules will settle the issue.

The pathway of receptor-induced PI hydrolysis has been reviewed extensively (142). Receptor triggering induces the action of phospholipase C and the consequent production of many different inositol phosphates and of diacylglycerol. Studies with $\text{GTP}\gamma\text{S}$ are consistent with a GTP binding protein being coupled to PI hydrolysis (143, 144). However, other studies show that it is not via a pertusis or cholera toxin-sensitive GTP binding protein (134, 145, 146). This leaves open the possibility that a GTP-binding protein such as G_z could link receptor triggering to PI hydrolysis.

Receptor triggering induces the action of phospholipases other than phospholipase C. Phospholipase A2 generates arachidonic acid, itself an intermediary in the synthesis of prostaglandins and leukotrienes (82). The receptor-mediated activation of phospholipase A2 seems to be mediated

by a GTP binding-protein (147). Phospholipase D is also activated to generate phosphatidic acid, which is then converted into diacylglycerol. In fact, this may be the major pathway of formation of diacylglycerol (148). In turn diacylglycerol is thought to activate protein kinase C. Receptor aggregation induces the membrane translocation of protein kinase C, but this may not be essential for exocytosis because depletion of protein kinase C does not abolish degranulation (149).

STRUCTURE OF THE FcR GENES AND MODEL FOR THEIR EVOLUTION

As members of the Ig supergene family, the FcR genes share features common to that family. As seen in Figure 2, the overall intron-exon organization of the Fc γ R α and Fc ϵ RI α genes is related, with each Ig domain encoded on a separate exon (38, 100, 150, 151). Similarly, all the genes described to date encode the leader peptide in two exons (S₁ and S₂). Distinctions among these genes can be seen in the exons encoding the transmembrane and cytoplasmic domains of the respective proteins, indicative of the functional divergence of these domains in FcR molecules. The Fc γ RIII α and Fc ϵ RI α genes demonstrate a single exon encoding these. Sequence homology is found among these genes in their leader peptide exons, their extracellular domain-encoding exons, and in the transmembrane sequences within the transmembrane-cytoplasmic exon. Not surprisingly, the subunit structures of the receptors encoded by Fc γ RIII and Fc ϵ RI are related (see below). While a single gene encodes the Fc γ RIII gene in the mouse, two highly homologous genes are found in the human. Greater than 95% sequence identity has been found for these genes, in both intron and exon sequences. However, single nucleotide substitutions in these genes result in significant differences in structure, function, and regulation. The IIIA α gene codes for a transmembrane protein that is associated with the γ and ζ subunits and is expressed only in macrophages and NK cells; the product of the IIIB gene is GPI anchored and only transcribed in neutrophils.

In contrast, the Fc γ RII genes encode the transmembrane domain and cytoplasmic domains in multiple exons. This exon organization results in the alternative splicing pattern of the mouse Fc γ RII and human IIIB genes, leading to the expression of alternative cytoplasmic domains for these spliced transcripts. Once again, a single gene encodes Fc γ RII in the mouse while three genes have been found in the human. Human Fc γ RIIB is the precise homologue of the mouse Fc γ RII gene, differing only in the size of its first cytoplasmic exon. An internal splice donor site, present in the mouse but not utilized, has been preferentially utilized in the human

Fc γ RIIB gene, resulting in an exon that has deleted half of this mouse sequence. The sequence differences include the loss of serine phosphorylation sites present in the mouse (see below). The functionally significant domain of this alternatively spliced IIB cytoplasmic exon, which is expressed in lymphocytes is likely to be the conserved amino terminal sequence.

The two additional genes encoding Fc γ RII proteins in the human, IIA and IIC, share overall intron-exon organization with the IIB gene. The difference between them and IIB occurs in the cytoplasmic exons. Although the first cytoplasmic exon of the IIB gene (C1) is intact in IIA and IIC with conserved splice donor and acceptor sequences, transcripts in which this exon is included have not been found, when tested by RT/PCR in a wide variety of lymphoid and myeloid cells. The second cytoplasmic exon is conserved and functional in these genes, while the third and final cytoplasmic exon is divergent. Sequences homologous to the IIB third cytoplasmic exon are apparent within the IIA and IIC genes, although they appear as noncoding sequences found in the 3' untranslated regions of the gene. This difference appears to result from a mutation in the splice acceptor site of this third cytoplasmic exon of the archetypal IIB gene (from AG to ATG), rendering it nonfunctional. A cryptic splice acceptor site, 5' of these sequences, has been utilized in the IIA and IIC genes, resulting in a novel cytoplasmic exon which encodes 75% of the intracellular domain of these proteins. Finally, IIA can be seen to be a chimeric gene, with sequence homology in its signal and first extracellular domains to the Fc γ RIII genes, while the remainder is homologous to the Fc γ RIIB and C genes. The protein products encoded by IIA and IIC are nearly identical and their pattern of expression overlaps, suggesting that they have similar functions. Consistent with this notion has been the isolation of an allele of IIC in which a point mutation in the first extracellular domain results in a translational termination codon, rendering it a pseudogene (38).

While structurally similar in its genomic organization to the other FcR genes, Fc γ RI demonstrates sequence homology to the other FcR genes only in its first two extracellular domains. Thus, while it is clearly related to these genes, Fc γ RI represents another class of FcR genes in this cluster (18).

Molecular cloning and chromosome mapping of the Fc γ R genes in both rodent and human cells have defined the significant features of this complex locus and how the human locus may have evolved. The region of chromosome 1q23 that encodes the FcRs (Fc γ RI, II & III; Fc ϵ RI α) is syntenic in the mouse, sharing a variety of flanking markers (151). It is reasonable to assume, therefore, that a common ancestor to all these molecules underwent gene duplication to give rise to Fc γ Rs and Fc ϵ RI α . Further dupli-

cation of the Fc γ R locus resulted in the Fc γ RII and Fc γ RIII. As described above, the human locus is considerably more complex. After an initial duplication of the ancestral Fc γ RII and Fc γ RIII-like genes, further duplication resulted in four genes: two Fc γ RII and two Fc γ RIII. Thus, the IIIA-IIB-IIIB-IIB cluster evolved away from this structure as a result of a mutation that occurred in the splice acceptor site of the third cytoplasmic exon of the II gene, rendering it nonfunctional. As noted above, utilization of a new splice acceptor site in sequences formerly intronic of the ancestral Fc γ RII gene resulted in the emergence of a new cytoplasmic exon for the gene now recognized as IIC, giving rise to a cluster of IIIA-IIA--IIC-IIIB-IIB. Recombination or gene conversion between IIC and III could have produced a chimeric gene with a Fc γ RIII-like signal sequence and Fc γ RII-like extracellular, transmembrane, and cytoplasmic domains, including the novel cytoplasmic exon of IIC. This chimeric gene is designated IIA and would result in the genes IIIA-IIA-IIC--IIIB-IIB. Cosmid and YAC cloning has established the linkage of IIIA with IIA, and IIIB with IIC and IIB (38).

At the very least this clustering of genes and duplication indicates selection to maintain a redundancy of genes in the human genome, perhaps to ensure the presence of functional Fc γ R. Possibly catastrophic loss of these receptors would be lethal to the organism; alternatively or in addition, duplication permitted the acquisition of diverse cellular functions for these receptors.

SUBUNITS ASSOCIATED WITH Fc γ RS AND Fc ϵ RI

Until recently Fc receptors were thought to consist of single polypeptide chains (3). The only exception was the well-described tetrameric Fc ϵ RI. The finding that mouse macrophage Fc γ RIII associate with γ chains identical to Fc ϵ RI γ was the first evidence that other Fc receptors might also be multimeric complexes (40). These associated γ subunits were missed because they easily dissociate in detergent, in a way similar to that for Fc ϵ RI (see above). Other yet undefined subunits may have been missed for a similar reason. This section reviews the status of our knowledge in this regard.

Fc ϵ RI β and β -Like Polypeptides

Fc ϵ RI β (see above) has not been found in other receptor complexes. The restrictive expression of transcripts for rodent β in mast cells and basophils is consistent with an exclusive association of β with Fc ϵ RI. However, it is possible that β -like polypeptides will be identified as part of other complexes. One candidate includes mouse Ly-44 or its human equivalent

CD20, although there has been no report of molecules associated with these selective B-cell markers. Like β , CD20 contains four presumptive transmembrane domains and has amino and carboxy termini protruding in the cytoplasm (152). Furthermore, 29% identical residues are found between mouse β (residue 45 to 141) and mouse Ly44 (residues 37 to 129). Since both mouse Fc ϵ RI β and Ly44 genes are closely associated on chromosome 19 (153), it is likely that these genes have evolved by duplication. It is tempting to speculate that other members of this family will be detected in the future and that other β -like proteins will be found in multimeric complexes, possibly in Fc receptor complexes.

A Family of Disulfide-Linked Dimers Shared by Fc Receptors and the T Cell Antigen Receptor (TCR)

Disulfide homodimers of γ chains were found initially as part of the Fc ϵ RI complex (see above). Recently, Northern blots identified transcripts for γ chains in cells that do not express Fc ϵ RI, for example in macrophages and NK cells (40, 60). Sequencing of cDNA clones encoding γ chains from mouse macrophages (40) and human NK cells (60) confirmed that these γ chains are truly identical to Fc ϵ RI γ . The high level of homology between the transmembrane domains of Fc ϵ RI α and of Fc γ RIII was intriguing (see above), there being eight consecutive identical amino-acid residues. For this reason, the possible association of γ chains with Fc γ RIII was investigated. The first evidence of this association came from transfections studies. Transfection of mouse Fc γ RIII gene into Cos-7 cells results in very little expression of Fc γ RIII on the cell surface (37). By contrast, efficient surface expression of Fc γ RIII is obtained upon cotransfection of mouse Fc γ RIII and Fc ϵ RI γ genes (40, 41). This complementation suggests an interaction between mouse Fc γ RIII and Fc ϵ RI γ . Furthermore, these two molecules copurify, demonstrating physical association between them (40). A similar direct physical association between human Fc γ RIIIA and Fc ϵ RI γ has not yet been demonstrated, but the gene for γ chain augments the surface expression of human Fc γ RIIIA α in transfected cells (41, 60). This implies a physical association of homodimeric Fc ϵ RI γ chains with both mouse Fc γ RIII α and human Fc γ RIIIA α .

Comparison of the genes for Fc ϵ RI(Fc γ RIII) γ (103) and TCR ζ chain (154–156) indicates that they belong to the same family and have been generated by duplication (103) (Figure 3). Both genes are located on mouse (153, 156, 157) and human chromosome 1 (155, 158) and show an analogous organization of their exons. In both genes, the leader peptide is encoded by two exons, the second of which also contains the short extracellular domain, the hydrophobic transmembrane region, and the beginning of the cytoplasmic tail. The following exons, exons 3–5 and



Figure 3 Comparison of the γ and ζ genes and proteins. Amino acid sequence comparison is divided into the corresponding exons encoded by each gene. Conserved residues are highlighted. The topology of the proteins are indicated.

exons 3–7 for γ and ζ , respectively, encode the remainder of the cytoplasmic tail. Furthermore, a high level of homology between the two genes is found in three of their respective exons, at the DNA and protein level (both about 50%). Finally, both γ and ζ polypeptides use homologous cysteines to form disulfide-linked dimers (105), have the same topology, and are essential for the surface expression of their respective receptors.

The detection of transcripts for ζ chains in TCR-, CD3- NK cells (159) led to the finding that human Fc γ RIIIA α from NK cells physically associates with ζ - ζ homodimer (65, 68) and with ζ - γ heterodimer (160). Like γ chains, human ζ (but surprisingly not mouse ζ) can also complement the surface expression of human Fc γ RIIIA α and of mouse Fc γ RIII α in transfected Cos-7 cells (41). Interestingly, human ζ can substitute for γ in assembly and in surface expression of Fc ϵ RI in xenopus oocytes (161).

So far three different dimers have been identified in Fc receptor complexes: γ - γ , ζ - ζ , and ζ - γ (Figure 1). These dimers are also part of the TCR complex (160) and probably mediate similar functions. There is a third member of the same family, TCR η which is generated by alternate splicing from the same gene as TCR ζ (162). The dimers η - η , η - ζ , and η - γ apparently are only associated with TCR, and so far there is no evidence that they associate with Fc receptor structures. Possibly, new members of the same family will be identified that form part of Fc receptor complexes.

CONCLUSIONS

Our knowledge of the complexity of the Fc receptor structures has increased dramatically over the last few years. Although Fc receptors share many features and belong to the same family, the diversity and heterogeneity of these receptors is greater than expected. Two of these receptors, Fc ϵ RI and Fc γ RIII, are now known to be multimeric complexes of noncovalently associated subunits. Possibly additional subunits will be discovered in association with these Fc receptors or as part of new Fc receptor complexes.

Functional questions remain to be resolved, particularly for the Fc γ Rs: for example, which receptor is responsible for a particular biological effect? Our knowledge regarding the role of individual subunits is fragmentary, as is our understanding of how FcRs mediate intracellular signalling. The challenge will be to map signal transducing pathways onto the appropriate molecules.

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NOTE ADDED IN PROOF

A cDNA clone encoding the human $F_c\alpha RI$ has been described [Moliszewski et al, *JEM* 172: 1665 (1990)], isolated from the monocyte-like cell line V937. The predicted amino acid sequence reveals homology to $F_c\gamma RI$ and III and $F_c\epsilon RI\alpha$ in the extracellular region, suggesting that it is derived from the same common ancestor as the other F_cRs . Transcripts for $F_c\alpha RI$ were detected in V937 cells, peripheral monocytes and granulocytes and were absent in lymphocytes.

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