

FcR γ Chain Deletion Results in Pleiotropic Effector Cell Defects

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Summary

The γ subunit of immunoglobulin Fc receptors is an essential component of the high-affinity receptor for IgE (Fc ϵ RI) and the low-affinity receptor for IgG (Fc γ RIII) and is associated with the high-affinity receptor for IgG (Fc γ RI) and the T cell receptor-CD3 complex. It is required for both receptor assembly and signal transduction. Targeted disruption of this subunit results in immunocompromised mice. Activated macrophages from γ chain-deficient mice unexpectedly lack the ability to phagocytose antibody-coated particles, despite normal binding. Defects in NK cell-mediated antibody-dependent cytotoxicity and mast cell-mediated allergic responses are evident in these animals, establishing the indispensable role of FcRs in these responses. However, loss of γ chain does not appear to perturb T cell development, since both thymic and peripheral T cell populations appear normal. These mice thus represent an important tool for evaluating the role of these receptors in humoral and cellular immune responses.

Introduction

Fc receptors (FcRs) for IgG and IgE couple humoral and cellular immunity by directing the interaction of antibodies with effector cells. These receptors are present on most effector cells of the immune system and mediate phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), activation of inflammatory cells, and many of the biological sequelae associated with antibody-dependent immunity (reviewed by Ravetch and Kinet, 1991; Beaven and Metzger, 1993). Intensive analysis of the genes and proteins encoded by this family of receptors has revealed a structural heterogeneity for these receptors that mirrors the functional diversity mediated by these cell surface molecules (Ravetch et al., 1986).

The high-affinity FcR for IgE, Fc ϵ RI, is found on mast cells and basophils and is responsible for the degranulation of these cells in response to cross-linking by antigen (reviewed by Parker, 1987). It is the receptor primarily responsible for triggering both peripheral and systemic anaphylaxis. In addition to the well-known pathological response of these cells when activated by allergen, Fc ϵ RI has been associated with host resistance to parasitic infec-

tions (Matsuda et al., 1990). In contrast with the restricted expression of Fc ϵ RI, FcRs for IgG, Fc γ Rs, are found on most cells of the hematopoietic lineage and mediate both high- and low-affinity binding to IgG. The high-affinity receptor, Fc γ RI, binds monomeric IgG and is expressed exclusively on macrophages and neutrophils. It is capable of mediating ADCC and phagocytosis in response to cross-linking by antibody (Askenase and Heyden, 1974; Heusser et al., 1977; Diamond et al., 1978). The low-affinity receptors for IgG, Fc γ RII and Fc γ RIII, are responsible for effector cell responses to immune complexes and represent the Fc γ Rs primarily involved in the inflammatory response in vivo. Fc γ RII is widely expressed on hematopoietic cells and functions as an inhibitory receptor on B cells (Uher et al., 1985; Kurosaki et al., 1994), while on cells of the myeloid lineage and on platelets, Fc γ RII triggers ADCC, phagocytosis, and the release of inflammatory mediators when cross-linked by immune complexes (Nathan et al., 1980). These disparate functions result from the genetic heterogeneity of Fc γ RII, as well as alternative splicing of its mRNA to generate proteins with distinct intracellular domains (Stuart et al., 1989; Brooks et al., 1989; Qiu et al., 1990). Fc γ RIII is restricted in its expression to natural killer (NK), macrophage, neutrophil, and mast cells, and mediates effector responses when cross-linked by immune complexes (Weinshank et al., 1988; Perussia et al., 1989). It is the sole FcR on NK cells, mediating all the antibody-dependent responses on those cells. In addition to these well-characterized effector cell pathways, Fc γ RIII has been found on immature (day 15) thymocytes, where it has been postulated to function in early thymocyte development (Rodewald et al., 1992).

Molecular characterization of the genes and protein products for Fc γ RIII and Fc ϵ RI revealed that these two receptors were homologous (Ravetch and Anderson, 1990; Ravetch and Kinet, 1991) and required the identical subunit, the γ chain, for efficient cell surface expression (Ra et al., 1989; Kurosaki and Ravetch, 1989). This homodimeric protein not only mediates assembly of these receptors by preventing the degradation of the ligand-binding α subunit in the endoplasmic reticulum (Weissman et al., 1989; Kurosaki et al., 1991), it is also critical for transducing signals into the cell interior, resulting in cellular activation through a tyrosine kinase-dependent pathway (Romeo and Seed, 1991; Wirthmueller et al., 1992). In murine macrophages, neutrophils, mast cells, and basophils, the γ chain is necessary for surface expression of Fc γ RIII and Fc ϵ RI. In NK cells, a homologous chain, the ζ chain, first described as a component of the TCR-CD3 complex, is also expressed and forms heterodimers with the γ chain (Kurosaki and Ravetch, 1989; Lanier et al., 1989). Based on reconstitution studies and in vitro experiments, the murine ζ chain alone cannot substitute for γ chain, owing to a single amino acid substitution in the transmembrane domain of this ζ sequence, replacing a leucine for an isoleucine (Kurosaki and Ravetch, 1989; Kurosaki et al., 1991). This change in the transmembrane

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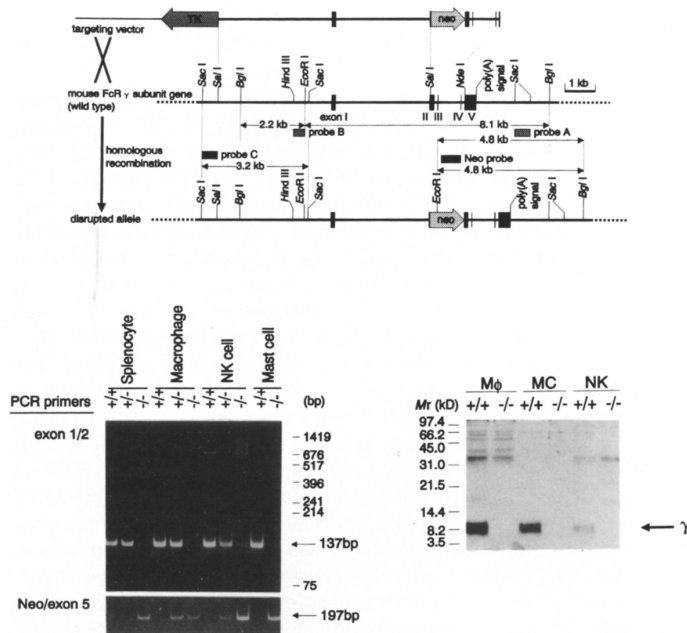


Figure 1. Targeted Disruption of the γ Chain and Characterization of γ Chain mRNA and Protein from FcR γ^{nl}

(Top) The γ subunit locus (wild-type allele), the targeting vector, and expected targeted allele are shown.

(Bottom left) Total RNA isolated from activated macrophages, mast cells, and NK cells and subjected to RT-PCR analysis, as described in Experimental Procedures. Amplified PCR products were electrophoresed on a 7.5% polyacrylamide gel. The sizes of amplified fragments are indicated in base pairs on right of each panel. +/+, wild type; +/-, heterozygote; -/-, homozygote.

(Bottom right) Western analysis of γ subunit expression. Cell suspensions were prepared from activated macrophages (M ϕ), mast cells (MC), and NK cells and subjected to Western analysis. A 6-9 kD γ subunit polypeptide is detected in cells derived from wild-type and heterozygous mice, but is absent in the homozygous mutant mice that produce no detectable γ subunit.

domain greatly diminishes the association of ζ chain with the ligand-binding α chain. The γ chain has also been found to be associated with the TCR-CD3 complex (Mercep et al., 1990), although its specific function distinguishing it from the homologous ζ chain has yet to be determined. Although not required for its surface expression or ligand binding in transfected fibroblasts, the γ chain has been found to be associated with Fc γ RI in the human monocytoid line U937 (Ernst et al., 1993), where it may function as a signal-transducing subunit.

The structural heterogeneity of Fc receptors for IgG, in which the IgG immune complex interacts with a diverse array of related receptors, and the overlapping pattern of FcR expression on effector cells have precluded detailed determination of the specific functions of individual receptors in mediating effector responses in vivo. We have created a mouse strain genetically deficient in the γ subunit by homologous recombination in ES cells in order to determine the roles of Fc γ RIII and Fc ϵ RI in effector responses to IgG and IgE, respectively. Selective ablation of this chain has indeed resulted in the loss of these receptors on NK cells, macrophages, and mast cells. The functional deficit, however, is more pronounced than would have been predicted from in vitro reconstitution studies alone. Inflammatory macrophages are unable to mediate phagocytosis through Fc γ RI, Fc γ RII, or Fc γ RIII, indicating an unexpected pleiotropic role of this subunit. These mice thus provide the first clear mutations with which we can determine the distinct roles of these structurally similar receptors in mediating effector responses in host defense.

Results

Targeted Disruption of the γ Subunit Gene in ES Cells

A targeting vector for the γ locus was constructed employing the poly(A) trap vector pMC1-neo (Thomas and Capecchi, 1987), which was inserted into the second exon of the γ subunit genomic subclone isolated from a 129/Ola λ library. This insertion created a new stop codon 239 bp downstream of the integration site. The γ subunit homologous recombination construct, pFCR γ P, contained 7.1 kb of homology 5' and 1.2 kb 3' of the neo insertion (see Figure 1).

The mutant allele generated by homologous disruption will be referred to as FcR γ^{nl} . Mouse chimeras were established from eight ES clones that had undergone homologous integration events. Two of seven male chimeras transmitted the mutant allele to their offspring. Homozygous FcR γ^{nl} animals were present at the expected frequency, indicating that disruption of the γ subunit locus did not result in embryonic lethality. The homozygous mutant mice appeared grossly normal and were fertile.

Verification of the FcR γ^{nl} Null Allele: RNA and Protein Analyses

Reverse transcription polymerase chain reaction (RT-PCR) analysis of macrophage, NK cell, and mast cell RNA isolated from wild-type, heterozygous, and homozygous mutant mice was performed to determine the level of expression of γ chain RNA. A 137 bp fragment specific for

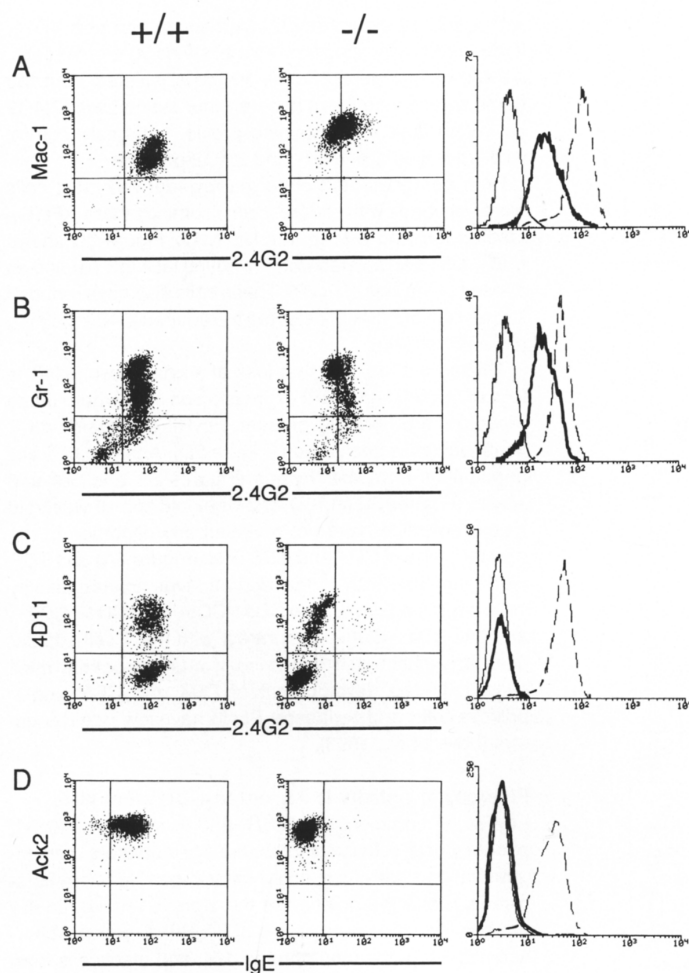


Figure 2. Flow Cytometric Analysis of Fc Receptor Expression on Effector Cells

(A) Fc γ RII and Fc γ RIII expression on TEMs. Macrophages were stained with 2.4G2 and the macrophage marker Mac-1; homozygous mutant mice have an 80% reduction in 2.4G2 staining (bold tracing).

(B) Fc γ RII and Fc γ RIII expression on bone marrow neutrophils. Neutrophils were obtained from the bone marrow and stained with 2.4G2 and the granulocyte-specific marker, Gr-1. In the homozygous mutant mice, 2.4G2 staining is reduced by 50% (bold tracing).

(C) Fc γ RIII expression on IL-2-induced splenic NK cells. NK cells were prepared from IL-2-activated splenocytes as described in Experimental Procedures and stained with 2.4G2 and the NK cell marker 4D11. In both wild-type and mutant mice, 4D11 is expressed on 50% of NK cells, while 2.4G2 staining is undetectable in mutant mice (bold tracing).

(D) Fc ϵ RI expression on bone marrow-derived mast cells. Cells were stained with FITC-IgE and the mast cell marker Ack2. IgE staining is lost in the mutant mice (bold tracing), which retain Ack2 staining. Identical results were obtained using mast cells purified from peritoneal lavage cells by Ficoll gradient centrifugation (data not shown). Light tracing corresponds to control antibody staining; bold tracing indicated $-/-$ staining, while broken line corresponds to $+/+$ staining of the x-axis antibody. (Identical results were observed for $+/-$ and $-/-$ mice in all cases [data not shown]).

a transcript containing exon 1 and 2 sequences, located upstream of the *neo* integration site, was observed in wild-type and heterozygous animals, whereas homozygous mutant mice had no detectable fragment in macrophages, and a faint band in mast and NK cells (Figure 1, bottom left). Similar results were obtained using primers specific for exons 1 and 5. PCR analysis using *neo* and exon 5 primers detected the expected 197 bp fragment in macrophages from homozygous as well as heterozygous animals (Figure 1, bottom left). Intact γ message could not be detected in RNA isolated from brain, thymus, heart, lung, spleen, or kidney (data not shown). Protein extracts were prepared from macrophages, NK, and mast cells and subjected to Western blot analysis with an anti- γ chain antibody. Figure 1 (bottom right) shows that the band of 6–9 kd expected for the γ subunit protein (Alcaraz et al., 1987) was present in cells from the wild type but not from those of homozygous mutants. No abnormal γ subunit cross-reacting proteins were detected in cells from homozygous mice even in a 6-fold longer exposure (data not

shown). We conclude that the targeted mutation resulted in a mutant allele that does not produce a γ subunit protein.

Flow Cytometric Analysis of Fc γ RIII and Fc ϵ RI Expression

To determine whether the γ chain disruption indeed resulted in the loss of expression of Fc γ RIII and Fc ϵ RI, macrophages, neutrophils, mast cells, and splenic NK cells were harvested from the γ -deficient mice and their wild-type or heterozygous littermates. Thioglycollate-elicited peritoneal macrophages (TEMs) were stained with 2.4G2, a monoclonal antibody (MAb) that recognizes both low-affinity Fc γ Rs (Fc γ RII and Fc γ RIII) and were counterstained with a macrophage marker, Mac-1. As seen in Figure 2A, macrophages derived from $+/+$ mice are positive for both 2.4G2 and Mac-1, while the $-/-$ mice show an 80% reduction in 2.4G2 staining, without a reduction in Mac-1 staining, suggesting the loss of Fc γ RIII, with retention of Fc γ RII on these macrophages.

Bone marrow-derived neutrophils were characterized

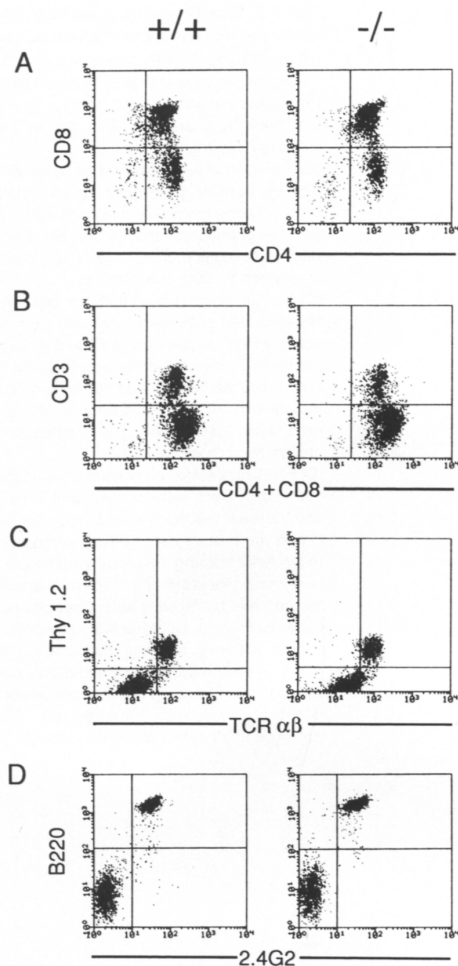


Figure 3. Flow Cytometric Analysis of Lymphocyte Populations in $Fc\gamma R^{\gamma-}$ Mice

(A and B) Expression of CD4–CD8 and CD4CD8–CD3 populations in the thymus of wild-type and mutant mice. Thymocytes or splenic T cells from either 2-week-old (data not shown) or 10-week-old mice were stained with MAb to CD4, CD8, and TCR chains. No differences are apparent in these populations in mutant mice when compared with the wild type.

(C) Expression of TCR $\alpha\beta$ in splenic T cells of mutant and wild-type mice.

(D) Expression of Fc γ RIII in splenic B cells of mutant and wild-type mice. The double-negative population represents splenic T cells not stained by B220 or 2.4G2.

by flow cytometry using 2.4G2 and a granulocyte marker, Gr-1. Wild-type (+/+) as well as heterozygous (+/-) (not shown) mice show a double-positive population of cells (Figure 2B); homozygous (-/-) mice have equivalent Gr-1 staining, with 2.4G2 staining reduced by 50%.

NK cells express only Fc γ RIII (Perussia et al., 1989); loss of γ chain would either result in the total ablation of surface expression of this Fc γ R or normal levels of expression if the ζ chain is able to substitute for the γ subunit efficiently. IL-2-stimulated splenic NK cells were stained

with 2.4G2 to detect Fc γ RIII expression and with 4D11, a murine NK cell-specific MAb that stains 50% of NK cells. As shown in Figure 2C, the NK cells isolated from the homozygous γ -deficient mice are not stained with 2.4G2 yet retain their 4D11 staining profile. Littermate control wild-type mice are 4D11- and 2.4G2-positive.

Both peritoneal and bone marrow-derived mast cells were stained with fluorescein isothiocyanate (FITC)-labeled IgE and a MAb to c-kit, Ack2. Figure 2D shows that mast cells isolated from -/- mice lack IgE binding as a result of the loss of Fc ϵ RI. These cells also have reduced expression of Fc γ RIII, detected by reduced 2.4G2 staining (data not shown).

These data indicate that loss of γ chain results in the loss of Fc γ RIII and Fc ϵ RI expression on cells where those receptors are normally present. Fc γ RII expression on B lymphocytes is unchanged (Figure 3D). Analysis of T cell populations from the thymus (Figures 3A and 3B) and spleen (Figures 3C and 3D) of 2-week-old and 10-week-old γ -deficient mice have not revealed any distortion in the normal ratios of CD4 and CD8 cells (Figure 3A) and TCR $\alpha\beta$ (Figure 3B). Both mutant and wild-type animals display 70% double-positive, 22% CD4+ CD8-, 6% CD4- CD8+, and 2% CD4- CD8-. In contrast with the T cell populations in γ chain-deficient mice, ζ chain knockout mice show impaired development of CD4 and CD8 single-positive cells, and peripheral T cells have few T cell receptors (Love et al., 1993).

Phagocytic Activity Is Absent in γ -Deficient Mice

Fc γ RIII, in common with Fc γ RI and II, mediates ADCC, phagocytosis, release of inflammatory mediators, and degranulation when cross-linked with antigen-antibody complexes. Since the deletion of the γ chain resulted in the marked reduction or total loss of Fc γ RIII, functional characterization of the macrophage, NK, and mast cells from these γ -deficient animals were studied to determine the contribution of Fc γ RIII to these physiological responses to IgG immune complexes.

FcR-mediated phagocytosis was assessed by the ability of TEMs to internalize sheep red blood cells (SRBCs) opsonized with IgG (Figure 4). IgG2a-opsonized RBCs are bound and internalized preferentially by the high-affinity Fc γ RI, while IgG1 and 2b are only bound and internalized by the low-affinity receptors (Weinshank et al., 1988). As shown in Figures 4A and 4E, macrophages from wild-type (+/+) mice displayed robust binding of IgG-opsonized SRBCs of G1 and 2a subclasses. Heterozygous mice displayed identical binding (data not shown), and IgG2b binding was comparable to IgG1 in all cases (data not shown). These bound opsonized particles were efficiently internalized, as shown in Figures 4B and 4F. Macrophages from homozygous mutant (-/-) mice bind IgG1-opsonized SRBCs (Figure 4C), owing to retained expression of Fc γ RII (see Figure 2A). This binding is completely blocked by MAb 2.4G2. These same macrophages failed to demonstrate IgG2a binding (Figure 4G), indicating an unexpected loss of Fc γ RI binding activity. This is not the result of loss of Fc γ RI α chain expression, since these macrophages express equivalent levels of mRNA for this chain

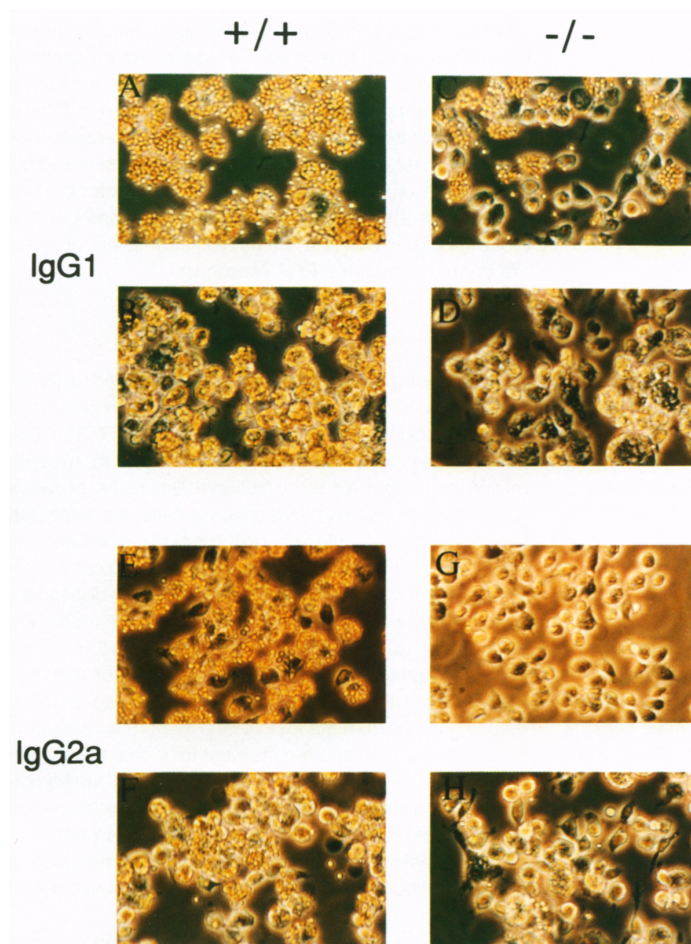


Figure 4. Rosetting and Phagocytosis of IgG-Opsonized Red Blood Cells by Activated Macrophages

Rosetting of IgG1-opsonized SRBCs by +/+ (A); phagocytosis of IgG1-opsonized SRBCs by +/+ (B); rosetting of IgG1-opsonized SRBCs by -/- (C); phagocytosis of IgG1-opsonized SRBCs by -/- (D); rosetting of IgG2a-opsonized SRBCs by +/+ (E); phagocytosis of IgG2a-opsonized SRBCs by +/+ (F); rosetting of IgG2a-opsonized SRBCs by -/- (G); phagocytosis of IgG2a-opsonized SRBCs by -/- (H).

when compared with wild-type mice (data not shown). Rather, it indicates a functional dependence of the high-affinity Fc γ RI for the γ chain either for facilitating surface expression or ligand binding for this receptor. Despite the efficient binding of SRBCs to -/- macrophages through Fc γ RIII, these cells fail to internalize such opsonized particles, indicating a more global defect in FcR-mediated phagocytosis. Deletion of γ chain thus has a pleiotropic effect on macrophage FcR-mediated ligand binding and phagocytosis beyond what might be expected from the loss of Fc γ RIII.

ADCC Activity, but Not Natural Killing, in NK Cells Was Severely Abrogated in Fc γ R^μ Mice

Natural killing activity, as measured against the YAC-1 tumor target, was normal for wild-type and mutant NK cells either freshly isolated from the spleen or purified over glass wool and cultured in IL-2 for 7 days (Figure 5A). The EL-4 target was less sensitive to NK lysis in both wild-type and mutant cell preparations. Deletion of γ chain thus has no effect on natural killing of tumor targets, consistent with the evidence that receptors other than Fc γ RIII mediate

this process. ADCC activity against TNP-derivatized and anti-TNP IgG1-coated EL-4 target, however, was markedly diminished in NK cells from γ -deficient mice, while wild-type NK cells showed clear ADCC activity against the same target cells; this ADCC activity was completely blocked by the MA b 2.4G2 (Figure 5B). These results indicate that ADCC activity is almost totally lost in NK cells in these mutant mice, owing to loss of functional Fc γ RIII expression.

Characterization of Mast Cell Functions

Mast cells were first sensitized with monoclonal mouse IgE and then triggered with monoclonal anti-mouse IgE antibody. Degranulation, [^{3} H]-serotonin release, IL-4 production, and prostaglandin D2 release of these cells were measured for wild-type as well as γ -deficient mice (Figure 6). As expected, degranulation and serotonin release from γ -deficient mast cells were only negligible above background, while +/+ mast cells responded well to IgE cross-linking (Figures 6A and 6B). Similarly, the amount of an IL-4-specific 267 bp cDNA fragment was markedly increased after stimulation of mast cells from wild-type mice

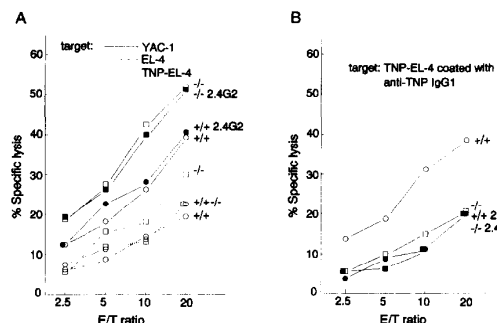


Figure 5. Natural Killing and ADCC Activities of Splenic NK Cells from Wild-Type and FcR γ^{Δ} Mice

Splenic NK cells generated after in vitro culture in the presence of exogenous IL-2 were tested for lytic activity against a set of targets (A) including the NK-sensitive target YAC-1, the thymoma EL-4, and the TNP-derivatized EL-4. (B) ADCC activities were tested on the following targets: TNP-derivatized EL-4 cells coated with an anti-TNP IgG1 antibody, or TNP-derivatized, anti-TNP-coated EL-4 cells in the presence of the anti-Fc γ RIII antibody 2.4G2.

(Figure 6C). In contrast, mast cells from γ -deficient mice showed apparently no response to cross-linking stimulation. Finally, prostaglandin D2 release into the culture supernatant was significantly reduced in mutant mast cells, as compared with wild-type cells (Figure 6D). Thus, by several criteria, assessing early and late activation responses of mast cells to IgE cross-linking, the mutant mice fail to respond.

IgE-Mediated Anaphylaxis Is Absent in FcR γ^{Δ} Mice

IgE-mediated anaphylaxis is thought to be dependent upon Fc ϵ RI triggering of mast cells, although the role of

other cell types and other IgE binding molecules to this in vivo response is debated. To address this question, we challenged mutant and wild-type mice in a passive cutaneous anaphylaxis (PCA) model as shown in Figure 7. The characteristic increase in vascular permeability triggered by mast cell degranulation is readily visualized by Evans blue extravasation. Wild-type and heterozygous mice mount a prompt PCA reaction in response to IgE cross-linking. In contrast, homozygous deficient animals are unable to mount a PCA reaction.

Discussion

Gene targeting in embryonic stem cells was used to generate a mouse strain carrying a mutation in the γ subunit of FcRs, a subunit also found associated with TCR-CD3. Mice homozygous for this mutation failed to express mRNA or protein for the γ subunit. Based on previous reconstitution studies in transfected cells, we expected that this mutation would result in the loss of surface expression for Fc γ RIII and Fc ϵ RI. Since these receptors are restricted in their cell-type expression, antibody-dependent effector pathways involving mast cells, macrophages, neutrophils, and NK cells would be expected to be affected. In addition, abnormalities in either T cell development or function might be manifested by these animals, resulting either from the loss of Fc γ RIII from an early thymocyte population or a requirement for γ chain as a component of TCR-CD3 in some T cells. Deletion of the homologous ζ chain does result in such T cell developmental abnormalities (Love et al., 1993). Homozygous γ -deficient mice are viable up to six months and fertile, demonstrating that the γ subunit is not required for normal mouse development.

In contrast with the absence of obvious developmental

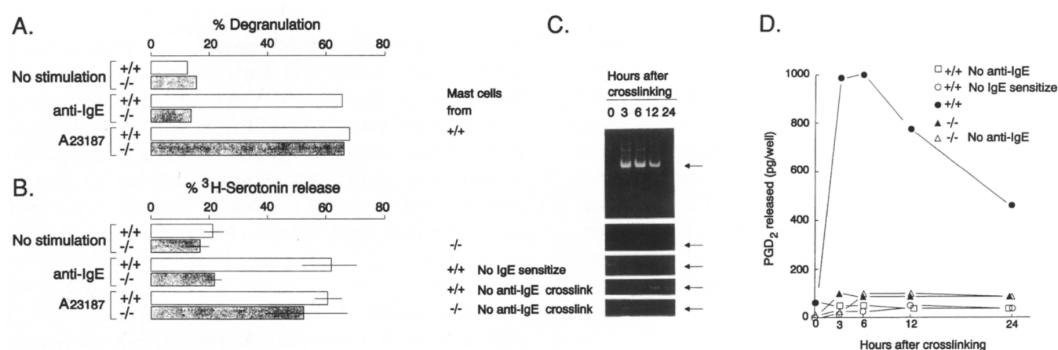


Figure 6. Functional Characterization of Mast Cells from Wild-Type and FcR γ^{Δ} Mice

(A) Mast cell degranulation. Bone marrow-derived mast cells were presensitized with monoclonal murine IgE and subsequently degranulated by cross-linking with murine anti-IgE antibody. Maximal degranulation was determined using ionophore A23187. (B) Mast cell serotonin release. Bone marrow-derived mast cells were preincubated with [³H]-serotonin and then incubated with monoclonal murine IgE. [³H]-serotonin released into the supernatant was quantitated after exposure to monoclonal anti-IgE or to ionophore A23187. (C) RT-PCR analysis of IL-4 mRNA after cross-linking of IgE on mast cells. Mast cells were prepared and sensitized as above, and cells were collected and subjected to RT-PCR analysis for IL-4 mRNA as described in Experimental Procedures. (D) Prostaglandin D2 release from mast cells after cross-linking of IgE. Bone marrow-derived mast cells were sensitized overnight with mouse monoclonal IgE. The cells were stimulated as above, and culture supernatants were collected at various time intervals. Amount of prostaglandin D2 released in the supernatant was determined by radioimmunoassay.

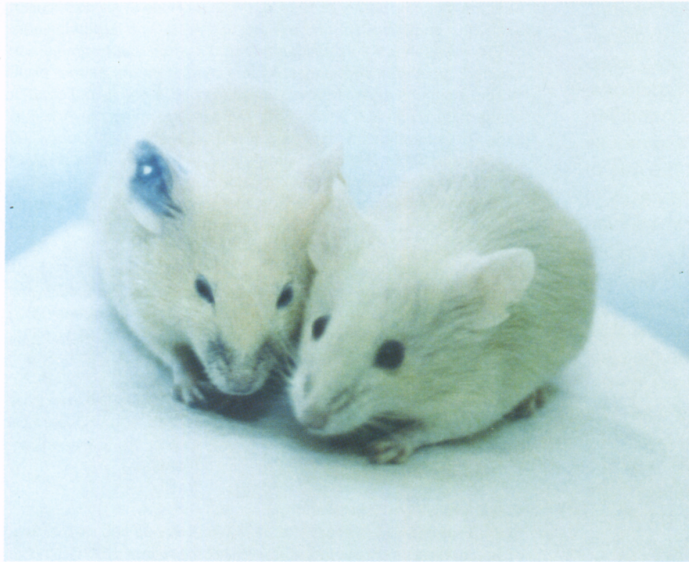


Figure 7. Passive Cutaneous Anaphylaxis in Wild-Type and FcR $\gamma^{-/-}$ Mice

γ -Deficient (right) and wild-type (left) mice were injected intradermally in the right ear with murine anti-DNP IgE; the left ear was injected with diluent alone. Subsequently, both mice were injected intravenously with DNP-HSA containing 1% Evans blue. IgE responsiveness, as manifested by increased vascular permeability and extravasation of blue dye, is evident in the IgE-primed right ear of the wild-type control, but not in the deficient mouse. Heterozygote responsiveness (data not shown) was identical to that of the wild-type mouse. Murine anti-DNP IgG did not prime for PCA (data not shown).

abnormalities, γ subunit-deficient mice clearly show several types of immunodeficiency, resulting from defects in FcR-mediated effector functions. While these experiments were undertaken to define precisely the role of each of these FcRs in antibody-mediated effector responses, the results of our studies revealed that these mice are more profoundly immunocompromised than could have been predicted from prior *in vitro* studies.

The data presented here reveal four marked differences between the mutant mice and their wild-type littermates. First, macrophages have lost their phagocytic activity against IgG-opsonized SRBCs. Although homozygous mutant mice did exhibit significant binding capacity to SRBCs opsonized with IgG1 and 2b subclasses of mouse immunoglobulin, as assessed by a rosetting assay, they did not show any activity to ingest them, indicating that homozygous mutant mice still have Fc γ RII, but the contribution of this receptor to macrophage phagocytic function is lost, owing to γ chain deletion. No evidence to date suggests that γ chain associates with this Fc γ RII (referred to as IIB), the only Fc γ RII gene found in the mouse and the only Fc γ RII gene conserved between mouse and man. IIB has been shown to undergo alternative splicing, generating proteins with variant cytoplasmic domains (Ravetch et al., 1986) that target the receptor to different intracellular compartments upon endocytosis of immune complexes (Miettinen et al., 1989). Macrophages express the IIB2 form of this gene, which localizes to coated pits, while B lymphocytes preferentially express IIB1. The failure of γ -deficient mice to mediate phagocytosis through IIB2 may suggest a functional coupling of this receptor to a common signaling subunit or indicate that IIB2 *in vivo* functions only as an inhibitory receptor, as has been demonstrated for B cells. Distinguishing between these two alternatives is now in progress.

Second, functional expression of Fc γ RI is greatly diminished on macrophages from γ -deficient mice. This receptor binds IgG2a with high-affinity and cannot be inhibited by a MAb directed against Fc γ RII and Fc γ RIII, 2.4G2. Mutant macrophages fail to bind IgG2a-coated SRBCs through this receptor, indicating a functional requirement for the γ subunit. Previous studies have demonstrated the ability of Fc γ RI to be expressed in heterologous cells, like COS7, in the absence of other subunits (Allen and Seed, 1989). The cell surface molecule expressed on those transfected cells retains its ability to bind IgG2a. Thus, the failure of macrophages isolated from γ -deficient mice to bind IgG2a through Fc γ RI was unexpected. Since no antibody reagents exist that recognize this murine receptor, we cannot determine whether γ chain is required for cell-surface expression of this receptor in macrophages or for ligand binding when expressed on its native cell. The observation that γ chain associates with human Fc γ RI in a myelomonocytic cell line suggests that the transfection results in COS7 cells do not mimic the native receptor in macrophages. Since Fc γ RI is functionally absent from the γ -deficient cells, the net result of γ deletion is an animal whose macrophages are unable to mediate phagocytosis of IgG-opsonized particles. These mice will be invaluable in determining the contribution of phagocytosis of IgG-opsonized pathogens in the normal immune response.

Third, freshly isolated or IL-2-stimulated NK cells from γ -deficient mice have lost the ability to mediate ADCC, while they retain natural cytolytic activity against tumor targets like YAC-1. This defect in ADCC is the result of the loss of expression of Fc γ RIII, since NK cells do not express any of the other known FcRs. These results eliminate any possibility of other surface components of NK cells substituting for Fc γ RIII in ADCC. The absolute requirement for γ chain expression in the mouse is to be

contrasted with the situation in human NK cells, where ζ chain can efficiently substitute for γ chain. Thus, mice can assemble only two types of Fc γ RIII, $\alpha\gamma_2$ and $\alpha\gamma\zeta$, while human NK cells have been shown to possess an additional receptor complex composed of $\alpha\zeta_2$. No functional differences have been observed between human and mouse NK cells in mediating ADCC, suggesting that the role of ζ chain is not critical to this response. No differences in the number of NK cells or their distribution were observed in these mutant mice, further indicating that the Fc γ RIII⁺ early thymocyte population defined previously (Rodewald et al., 1992) is not required for NK cell development.

Fourth, mast cells from γ -deficient mice have lost their response to IgE cross-linking and fail to mediate the classic allergic responses of mast cells. These cells do not degranulate, secrete prostaglandin D₂, induce IL-4 synthesis, or release serotonin upon IgE stimulation and cross-linking. When these mice are challenged in a PCA assay, they fail to mount a response, further indicating the significance of this receptor in mediating the anaphylactic response. These functional defects in mast cells are likely to be the direct result of the loss of Fc ϵ RI expression on those cells. While we cannot rule out the possibility that concomitant loss of Fc γ RIII from mast cells may contribute to this deficiency, it is unlikely to play a significant role, since that receptor has been shown to mediate only low-affinity binding to IgE. That the γ chain may contribute to these mast cell defects in a pleiotropic manner, affecting receptors other than Fc ϵ RI, similar to what we have observed in macrophages, remains a possibility that can be resolved by comparing FcR γ^{fl} mice with those whose loss of Fc ϵ RI results from the deletion of the ligand-binding α subunit of this receptor. In those mice, the same functional defects are seen (J.-P. Kinet, personal communication), thus firmly establishing the essential role of Fc ϵ RI in mediating mast cell responses to IgE and in vivo anaphylaxis.

In marked contrast with the defects in innate immunity resulting from FcR perturbation, T cell development appears to be grossly normal. The same populations of cells were observed in thymocytes and peripheral T cells from 2-week- or 10-week-old mice in the γ -deficient animals and their wild-type littermates. This chain, while found associated with TCR-CD3 in some populations of T cells, is clearly not critical for normal T cell development, in contrast with what has been reported in ζ -deficient animals. This preliminary analysis of T cell populations in these mutant mice further suggests that the dominant Fc γ RIII⁺ population of early thymocytes (days 14.5 through 16.5) is not essential for progression through the normal T cell developmental program. Whether specific defects in T cell populations that appear to use the γ chain exclusively are found in these animals remains to be determined.

These mice therefore will enable studies to be performed evaluating the contribution of IgG- and IgE-triggered effector responses to a variety of pathogens and will aid in further defining the role of this subunit in both T cell and effector cell pathways.

Experimental Procedures

Construction of the Targeting Vector pFCR γ P

Phage clones spanning the mouse FcR γ subunit gene were isolated

from the genomic library of 129/Ola origin (te Riele et al., 1990) using a mouse γ subunit cDNA probe (Ra et al., 1989). The targeting vector pFCR γ P was made by subcloning a 1.1 kb XhoI-SalI fragment containing 5' upstream sequences, exon 1, and 5' part of exon 2 of the murine γ subunit gene into the XhoI site of pMC1-*neo* (Stratagene) to create pMC1- γ -*neo*. A 3.9 kb SalI fragment spanning the 3' part of exon 2 to sequences 5' of exon 5 of the γ gene was subcloned into the SalI site of pMC1- γ -*neo* to create pMC1-*neo*-FCR γ . The resulting insert was integrated into the XhoI site of pC19R-MC1k (Marsh et al., 1984; Thomas and Capecchi, 1987) to give pFCR γ P. The vector was linearized at a unique ClaI site within the polylinker of the plasmid.

Monoclonal Antibodies

The following primary antibodies were used in this study: 2.4G2 (anti-Fc γ RIII/III, Unkeless, 1979); TIB191 (anti-TNP IgG1 from the ATCC); IGEL (anti-TNP IgE from the ATCC); anti-SRBC IgG2a (S-S.1), IgG2b (N-S.8.1), and IgG3(NS.7), all obtained from the ATCC; RM4-5 (anti-CD4), 53-6.7 (anti-CD8), H57-597 (anti-TCR $\alpha\beta$), DNL-1.9 (anti-B220), RB6-8C5 (anti-granulocyte), and 145-2C11 (anti-CD3), all from Pharmingen; M1/70 (anti-Mac-1) and 30-H12 (anti-Thy1.2), from Boehringer Mannheim; 4D11 (anti-LGL-1), kindly provided by Dr. L. Mason; and Ack2 (anti-c-kit), kindly provided by Dr. S. Nishikawa.

Cell Cultures and Transfections

The ES cell line E14 (Hooper et al., 1987) was maintained as described on feeder layers from primary embryonic fibroblasts in a 37°C, 5% CO₂ humidified incubator. ES cells (1×10^3) in 600 μ l of phosphate-buffered saline (PBS; Ca²⁺- and Mg²⁺-free) containing 50 μ g of linearized targeting vector were electroporated at 800 V with a 3 μ F capacitance in a 0.4 cm wide cuvette (Bio-Rad Gene Pulser). After approximately 10 min, the cells were plated on 60 mm dishes with *neo*-resistant embryonic fibroblast feeder layer cells. Selection was started 24 hr later with 300 μ g/ml G418 together with 0.2 μ M FIAU.

Embryo Injection and Mouse Breeding

C57BL/6 blastocysts (3.5 days postcoitum) were flushed from the uterus of naturally mated females into DMEM containing 10% FCS. ES cells (10–20 cells) from each clone were microinjected into the blastocoel of each blastocyst, and the blastocysts were reimplanted into the uterus of day 2.5 pseudopregnant foster mothers. The E14 ES cell line was originally derived from an XY blastocyst of the 129/Ola strain (Hooper et al., 1987). Chimeric animals were then detected by the presence of agouti patches on the non-agouti (black) fur and were mated with non-agouti C57BL/6 animals. Germline transmission was scored by the presence of agouti offspring in the litter. Agouti F₁ offspring from chimera and C57BL/6 crosses were genotyped by genomic blotting of DNA prepared from tail biopsies. Animals heterozygous for the FcR γ^{fl} mutation were crossed to heterozygous siblings. F₂ offspring from these crosses were genotyped by genomic blotting to distinguish heterozygotes from homozygotes.

Southern Blot Analysis and Genotyping of Progeny

DNA was isolated from the E14 cell line and from each of the G418 and FIAU double-resistant ES cell clones. DNA was subjected to restriction enzyme digestion with BglI and EcoRI. Each digest was fractionated on a 0.7% agarose gel, transferred to GeneScreen (DuPont), and hybridized with 0.5 kb SacI fragment (see Figure 1, top). In addition, blots were also screened with a *neo*-HSV-*tk* probe, 0.34 kb SacI-SalI fragment (probe C), and 0.33 kb HindIII-EcoRI fragment (probe B) from the γ subunit genomic clone (see Figure 1, top).

Flow Cytometric Analysis

Single-cell suspensions from bone marrow, spleen, thymus, and peritoneal cavity of mice from 2 weeks to 3 months of age were prepared as described below. Aliquots of 10^6 cells were stained for 30 min at 4°C with FITC- or PE-conjugated antibodies as noted in the text. Unconjugated MABs were revealed by FITC- or PE-conjugated goat anti-mouse IgG F(ab). Cells were washed twice in PBS containing 1% BSA and 0.1% sodium azide after each incubation and fixed in PBS, 0.1% sodium azide, and 1% formaldehyde. Fluorescence intensity was measured on a FACScan flow cytometer using FACScan research software (Becton-Dickinson and Company). Dead cells were eliminated from the analysis on the basis of forward and sideways light scatter.

Preparation of IL-2-Activated Splenocytes

Spleens of wild-type and mutant mice were removed aseptically and crushed with the hub of a syringe and a stainless steel tea filter in medium (NK medium) consisting of RPMI1640 with 0.1 mM nonessential amino acids and 1 mM sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM glutamine, and 10% heat-inactivated FCS (HyClone). The whole spleen cell suspension was passed through stainless steel mesh, the erythrocytes lysed by Tris-NH₄Cl, and the remaining cells centrifuged and washed with NK medium and then passed through a nylon wool column. The nylon-passed cells were resuspended at 5×10^6 cells/ml and cultured for 4–7 days in NK medium supplemented with 1000 U/ml recombinant IL-2 (Gibco-BRL) and 1 μ g/ml indomethacin (Sigma). Fresh medium was added at a 1:1 volume every 3–4 days. The cells were harvested at the end of the incubation, washed, and used for the flow cytometric analysis or ^{51}Cr release cytotoxicity test.

RT-PCR Analysis

Total RNA was prepared from single-cell suspensions according to Chomczynski and Sacchi (1987) using RNeasy (Qiagen/Biotech Laboratories, Houston, Texas). First-strand cDNA synthesis from total RNA derived from 5×10^6 cells was performed using SuperScript reverse transcriptase (Gibco-BRL) and oligo(dT) primers. Oligonucleotide primers complementary to regions of exons 1 and 5 (Figure 1) were incubated with one twentieth of reverse-transcribed RNA mixture and subjected to 30 cycles of amplification using standard PCR protocols. PCR products were analyzed on a 7.5% polyacrylamide gel and visualized with ethidium bromide staining. Primer sequences for PCR amplification were as follows: 5'-GAGATCATCGGCATT-TTGAAC-3' and 5'-CTTGGACTCATTCATGGTGCA-3' for IL-4 cDNA (Noma, 1986; Lee, 1986), generating a 267 bp fragment; 5'-CCAGGATGATCTCAGCCG-3' and 5'-ACAGTAGAGTAGGGTAAG-3' for γ subunit cDNA corresponding to exons 1 and 2, generating a 137 bp fragment; 5'-CTTCCTCGTGCCTTACGGTATC-3' and 5'-CTTCAGAGTCTCATATGT-3' for *neo*- γ -subunit fusion cDNA (Figure 1), generating a 197 bp fragment.

IL-4 mRNA Induction and Prostaglandin D₂ Release from Mast Cells

Bone marrow cells were flushed out of the femurs and tibias of mice using a 23-gauge needle, resuspended, and then washed in PBS. The cells were cultured in MC medium consisting of 10% WEHI-3A culture supernatant or recombinant IL-3, 10% heat-inactivated FCS, RPMI1640 medium, 4 mM glutamine, nonessential amino acids, 10^{-5} M 2-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μ g/ml streptomycin, replacing the medium every 3–4 days. The bone marrow-derived, IL-3-induced mast cells were suspended at 5×10^6 cells/ml in MC medium and mixed with .25 vol of culture supernatant of mouse anti-TNP IgE-producing hybridoma, IGEL, at 37°C for 18 hr in 95% air, 5% CO₂. The cells were washed, resuspended at 4×10^6 cells/ml, and divided into 24-well culture plates at 1 ml/well with anti-mouse IgE MAb at 10 μ g/ml. The cells and culture supernatant were collected at various time intervals and stored at -80°C until use. The cell pellet was used for RT-PCR assay to determine IL-4 mRNA induction (Plaut et al., 1989), and culture supernatant was subjected for the radioimmunoassay of prostaglandin D₂ (Amersham).

Degranulation and [^3H]-serotonin release assay were performed using monoclonal mouse IgE as sensitizing antibody and anti-mouse IgE as stimulating antibody, as previously described (Daeron et al., 1992). Briefly, BM-derived mast cells (1×10^6) were incubated overnight with mouse IgE MAb at 4 μ g/ml and washed. In the serotonin release assay, they were incubated with 5 μ Ci/ml [^3H]-serotonin for 14 hr at 37°C and washed extensively. Cells were then stimulated with anti-mouse IgE MAb at 10 μ g/ml and incubated for 60 min. Maximal release was determined using A23187 as a nonspecific ionophore.

Macrophage Cultures

Mice were injected intraperitoneally with 1 ml of 5% thioglycollate, and peritoneal exudate cells were harvested 3 days later. The cells were suspended in alpha-modified MEM (α -MEM), supplemented with 10% heat-inactivated FCS, to a concentration of 1×10^6 cells/ml. They were plated in 24-well culture plates at 1 ml per well and incubated for 6 hr at 37°C in 95% air, 5% CO₂. Following this, the nonadherent

cells were removed by rinsing the monolayers with PBS, and the purified macrophages were subjected to the assays described below.

Preparation of Opsonized Erythrocytes, Rosetting, and Phagocytosis Assays

SRBCs (Gibco) were first derivatized with the hapten trinitrophenyl (TNP) as described (Rodewald et al., 1992). They were then incubated with the culture supernatant of TIB191 (anti-TNP IgG1). Alternatively, intact SRBCs were incubated with culture supernatant of hybridomas S-S.1, N-S.8.1, and N-S.7 (anti-SRBC IgG2a, 2b, and IgG3, respectively). These antibodies for opsonization were used at nonagglutinating titers. Opsonized SRBCs were added to macrophage monolayers prepared as described above and incubated either for 30 min at 4°C for determination of rosetting or for 90 min at 37°C in 95% air, 5% CO₂, and then washed extensively with PBS for determination of phagocytosis. For estimation of phagocytosis activity, they were further washed in distilled water to lyse hypotonically SRBCs rosetted extracellularly. The cells were then fixed in 0.25% glutaraldehyde and were photographed or counted for the number of rosetted and phagocytic cells using phase contrast microscopy.

Cytotoxicity Assays

Cytotoxicity assays using IL-2-induced splenic NK cells were performed in 96-well U-bottom plates (Corning), according to Rodewald et al. (1992). Target cells were labeled with ^{51}Cr (DuPont-NEN, Boston, Massachusetts) at 100 μ Ci per 10^6 cells for 1 hr at 37°C, washed twice, and used in a standard 4 hr ^{51}Cr release assay with 5×10^3 target cells per well. Maximum isotope release was measured by incubating the target cells in 1% Nonidet P-40. Spontaneous release was measured by incubation of the target cells in culture medium alone. Results are expressed as the mean of percent-specific lysis of triplicate samples (with SD $\leq 10\%$). NK activity was evaluated using various effector to target ratios against either the conventional NK target cell line YAC-1 (murine B lymphoma) or EL-4 (murine thymoma). For ADCC assays, EL-4 cells were ^{51}Cr labeled, treated with 30 mM 2,4,6-trinitrobenzene sulfonic acid (ICN Biochemicals) in PBS for 5 min at room temperature, washed, and incubated for 30 min with an anti-TNP antibody (IgG1, clone 1B7.11 [ATCC, Rockville, Maryland]) using culture supernatant (1:128 final concentration). Cells were washed extensively and used as targets as above. For the blocking of Fc γ RII/III function, effector NK cells were incubated with 2.4G2 at 10 μ g/ml for 30 min prior to the cytotoxicity assays; the blocking antibody was not removed before the assay. TNP derivatization or treatment with an anti-TNP antibody had no effect on spontaneous release of radioactivity.

Immunoblot Analysis

Cells (4×10^6) of TEM, IL-3-induced bone marrow mast cells, and IL-2-induced splenic NK cells were suspended in 30 μ l of gel-loading buffer, and 15 μ l (2×10^6 cell equivalents) was loaded on a 15% SDS-polyacrylamide gel. Following electrophoresis, protein was transferred to nitrocellulose for 18 hr at 20 V, and the blot was blocked 1 hr in PBS containing 5% nonfat milk powder and 0.1% Tween 20. FcR γ subunit was detected by incubating the blot first with the rabbit anti-mouse γ chain antiserum (Kurosaki and Ravetch, 1989), then washed extensively in PBS containing 0.1% Tween 20 and incubated with horseradish peroxidase-conjugated anti-rabbit antibody in PBS plus 0.1% Tween 20. After washing excess antibody away, the filter was developed using the Enhanced Chemiluminescence (ECL) detection system (Amersham).

Passive Cutaneous Anaphylaxis

Eight-week-old γ -deficient mice and their littermate controls were lightly anesthetized and injected intradermally in the left ear with 20 μ l of monoclonal mouse anti-DNP IgE (Sigma) diluted in 20 μ l of DMEM; the right ear was injected with DMEM alone. The next day, the mice were injected intravenously with 100 μ g of DNP-human serum albumin (Sigma) in 100 μ l of 0.9% NaCl; Evans blue dye (1%; Sigma) was added to permit visual localization of increased vascular permeability. The reaction was quantitated at 60 min post injection.

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References

- Alcaraz, G., Kinet, J.-P., Liu, T. Y., and Metzger, H. (1987). Further characterization of the subunits of the receptor with high affinity for immunoglobulin E. *Biochemistry* 26, 2569–2575.
- Allen, J. M., and Seed, B. (1989). Isolation and expression of functional high-affinity Fc receptor complementary DNAs. *Science* 243, 378–380.
- Askenase, P. W., and Heyden, B. J. (1974). Cytophilic antibodies in mice contact-sensitized with oxazolone. Immunohistochemical characterization and preferential binding to a trypsin-sensitive macrophage receptor. *Immunology* 27, 563–576.
- Beaven, M. A., and Metzger, H. (1993). Signal transduction by Fc receptors: the Fc ϵ RI case. *Immunol. Today* 14, 222–226.
- Brooks, D. G., Qiu, W. Q., Luster, A. D., and Ravetch, J. V. (1989). Structure and expression of human IgG Fc γ RII (CD32). *J. Exp. Med.* 170, 1369–1386.
- Chomczynski, P., and Sacchi, N. (1987). Single step method of RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162, 156–159.
- Daeron, M., Bonnerot, C., Latour, S., and Fridman, W. H. (1992). Murine recombinant Fc γ RIII, but not Fc γ RII, triggers serotonin release in rat basophilic leukemia cells. *J. Immunol.* 149, 1365–1373.
- Diamond, B., Bloom, B. R., and Scharff, M. D. (1978). The Fc receptors of primary and cultured phagocytic cells studied with homogeneous antibodies. *J. Immunol.* 121, 1329–1333.
- Ernst, L. K., Duchemin, A. M., and Anderson, C. L. (1993). Association of the high-affinity receptor for IgG (Fc γ RI) with the γ subunit of the IgE receptor. *Proc. Natl. Acad. Sci. USA* 90, 6023–6027.
- Heusser, C. H., Anderson, C. L., and Grey, H. M. (1977). Receptors for IgG: subclass specificity of receptors on different mouse cell types and the definition of two distinct receptors on a macrophage cell line. *J. Exp. Med.* 145, 1316–1327.
- Hooper, M., Hardy, K., Handyside, A., Hunter, S., and Monk, M. (1987). HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. *Nature* 326, 292–295.
- Kurosaki, T., and Ravetch, J. V. (1989). A single amino acid in the glycosyl phosphatidylinositol attachment domain determines the membrane topology of Fc γ RIII. *Nature* 342, 805–807.
- Kurosaki, T., Gander, I., and Ravetch, J. V. (1991). A subunit common to an IgG Fc receptor and the T cell receptor mediates assembly through different interactions. *Proc. Natl. Acad. Sci. USA* 88, 3837–3841.
- Kurosaki, T., Muta, T., Sanchez, M., Misulovin, Z., Nussenzweig, M., and Ravetch, J. V. (1994). A 13 amino acid motif in the cytoplasmic domain of Fc γ RIIB modulates B cell receptor signalling. *Nature*, in press.
- Lanier, L. L., Yu, G., and Phillips, J. H. (1989). Co-association of CD3 γ with a receptor (CD16) for IgG Fc on human natural killer cells. *Nature* 342, 803–806.
- Lee, F. (1986). Isolation and characterization of a mouse interleukin cDNA clone that expresses B cell-stimulating factor 1 activities and T cell- and mast cell-stimulating activities. *Proc. Natl. Acad. Sci. USA* 83, 2061–2065.
- Love, P. E., Shores, E. W., Johnson, M. D., Tremblay, M. L., Lee, E. J., Grinberg, A., Huang, S. P., Singer, A., and Westphal, H. (1993). T cell development in mice that lack the γ chain of the T cell antigen receptor complex. *Science* 261, 918–921.
- Marsh, J. L., Erfle, M., and Wykes, B. J. (1984). The pC plasmid and phage vectors with versatile cloning sites for recombinant selection by insertional inactivation. *Gene* 32, 481–485.
- Matsuda, H., Watanabe, N., Kiso, Y., Hirota, S., Ushio, H., Kannan, Y., Azuma, M., Koyama, H., and Kitamura, Y. (1990). Necessity of IgE antibodies and mast cells for manifestation of resistance against larval *Haemophysalis longicornis* ticks in mice. *J. Immunol.* 144, 259–262.
- Merćep, M., Weissman, A. M., Frank, S. J., Klausner, R. D., and Ashwell, J. D. (1990). Activation-driven programmed cell death and T cell receptor ζ η expression. *Science* 246, 1162–1165.
- Miettinen, H. M., Rose, J. K., and Mellman, I. (1989). Fc receptor isoforms exhibit distinct abilities for coated pit localization as a result of cytoplasmic domain heterogeneity. *Cell* 58, 317–326.
- Nathan, C. F., Murray, H. W., and Cohn, Z. A. (1980). The macrophage as an effector cell. *N. Engl. J. Med.* 303, 622–626.
- Noma, Y. (1986). Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. *Nature* 319, 640–646.
- Parker, C. W. (1987). Lipid mediators produced through the lipoxygenase pathway. *Annu. Rev. Immunol.* 5, 65–84.
- Perussia, B., Tutt, M. M., Qiu, W. Q., Kuzel, W. A., Tucker, P. W., Trinchieri, G., Bennet, M., Ravetch, J. V., and Kumar, U. (1989). Murine natural killer cells express functional Fc γ receptor II encoded by the Fc γ R α gene. *J. Exp. Med.* 170, 73–86.
- Plaut, M., Pierce, J. H., Watson, C. J., Janley-Hyde, J., Nordan, R. P., and Paul, W. E. (1989). Mast cell lines produce lymphokines in response to cross-linkage of Fc ϵ RI or to calcium ionophores. *Nature* 339, 64–67.
- Qiu, W. Q., de Bruin, D., Brownstein, B. H., Pearce, R., and Ravetch, J. V. (1990). Organization of the human and mouse low-affinity Fc γ R genes: duplication and recombination. *Science* 248, 732–735.
- Ra, C., Jouvin, M.-H., Blank, U., and Kinet, J.-P. (1989). A macrophage Fc γ receptor and the mast cell receptor for IgE share an identical subunit. *Nature* 341, 752–754.
- Ra, C., Jouvin, M.-H. E., and Kinet, J.-P. (1989). Complete structure of the mouse mast cell receptor for IgE (Fc ϵ RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem.* 264, 15323–15327.
- Ravetch, J. V., and Anderson, C. L. (1990). Fc γ R family: proteins, transcripts, and genes. In *Fc Receptors and the Action of Antibodies*, H. Metzger, ed. (Washington, D. C.: American Society for Microbiology), pp. 211–235.
- Ravetch, J. V., and Kinet, J.-P. (1991). Fc receptors. *Annu. Rev. Immunol.* 9, 457–492.
- Ravetch, J. V., Luster, A. D., Weinshank, R., Kochan, J., Pavlovic, A., Portnoy, D. A., Hulmes, J., Pan, Y.-C. E., and Unkeless, J. C. (1986). Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. *Science* 234, 718–725.
- Rodewald, H.-R., Moingeon, P., Lucich, J. L., Dosio, C., Lopez, P., and Reinherz, E. L. (1992). A population of early fetal thymocytes expressing Fc γ RII/III contains precursors of T lymphocytes and natural killer cells. *Cell* 69, 139–150.
- Romeo, C., and Seed, B. (1991). Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. *Cell* 64, 1037–1046.
- Stuart, S. G., Simister, N. E., Clarkson, S. B., Shapiro, M., and Mellman, I. (1989). The low-affinity Fc receptor for human IgG (hFcRII) exists as multiple isoforms. *EMBO J.* 8, 3657–3666.
- te Riele, H., Maandag, E. R., Clarke, A., Hooper, M., and Bens, A. (1990). Consecutive inactivation of both alleles of the *pim-1* proto-oncogene by homologous recombination in embryonic stem cells. *Nature* 348, 649–651.
- Thomas, K. R., and Capecchi, M. R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* 51, 503–512.
- Uher, F., Lamers, M. C., and Dickler, H. B. (1985). Antigen-antibody complexes bound to B-lymphocyte Fc γ receptors regulate B-lymphocyte differentiation. *Cell. Immunol.* 95, 368–379.

Unkeless, J. C. (1979). Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. Exp. Med.* 150, 580–596.

Weinshank, R. L., Luster, A. D., and Ravetch, J. V. (1988). Function and regulation of a murine macrophage-specific IgG Fc receptor, Fc γ R- α . *J. Exp. Med.* 163, 1909–1925.

Weissman, A. M., Baniyash, M., Hou, D., Samelson, L. E., Burgess, W. H., and Klausner, R. D. (1989). Molecular cloning of the zeta chain of the T cell antigen receptor. *Science* 239, 1018–1021.

Wirthmueller, U., Kurosaki, T., Murakami, M. S., and Ravetch, J. V. (1992). Signal transduction by Fc γ RIII (CD16) is mediated through the γ chain. *J. Exp. Med.* 175, 1381–1390.

Note Added in Proof

The data referred to throughout as J.-P. Kinet, personal communication, are now in press: Dombrowicz, D., Flamand, V., Brigman, K. K., Koller, B. H., and Kinet, J.-P. (1993). Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor α chain gene. *Cell* 75, 969–976.