A Novel Role for the Fc Receptor γ Subunit: Enhancement of FcγR Ligand Affinity

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Summary

The Fc receptors (FcR), which belong to the immunoglobulin (Ig) superfamily, bind to specific Ig isotypes with varying affinities triggering complex immune defense responses. Several of the FcR that lack signaling motifs in their cytoplasmic domains rely on associated subunits to transmit signals. Two classes of FcR that bind the Fc portion of IgG, FcγRI, and FcγRIIIa associate with a subunit shared among several FcR, the γ chain, which is involved in receptor expression and signal transduction. In this report, we propose that a novel role for γ chain is to enhance the affinity of FcγR for ligand. Our findings demonstrate that FcγRI requires γ-chain association to attain high affinity binding for monomeric IgG, and suggest that the intermediate binding affinity of the FcγRIIIa isoform results from its association with γ chain. The affinity increase conferred by γ chain appears to be mediated through the transmembrane domain of the FcγR, with no requirement for the cytoplasmic domain of the receptor.

The Fc receptors (FcR)[1], which bind the Fc region of Ig, provide an important link between cellular and humoral branches of the immune system by triggering several immune responses including phagocytosis, endocytosis, antibody-dependent cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation upon immune complex–mediated clustering (1, 2). Three classes of receptors for IgG (FcγR) have been identified—FcγRI, FcγRII, and FcγRIII—and all but one are integral membrane proteins constituted of glycosylated extracellular Ig-like domains, a short transmembrane domain, and a cytoplasmic domain. FcγRIIIb is not a transmembrane receptor, since it is anchored to the membrane by a glycosyl phosphatidylinositol (GPI) link. FcγRI is distinguished from the other FcγR members in having a unique third extracellular domain, while FcγRII and FcγRIII are two extracellular domain receptors. FcγRI and FcγRIIIa both associate with γ chain, a 20-kD disulfide-linked, homodimeric, signal-generating subunit that also associates with the high affinity IgE FcR (FcεRI) and the IgA FcR (FcαR) (3–5). In addition to functioning as a signal-transducing subunit, γ chain also plays a role in the cell-surface expression of some of these receptors; for example, FcεRI and the FcγRIIIa isoform require association with γ chain for expression in COS cells (6, 7). Expression of FcγRI and FcαR in eukaryotic cells, however, is independent of γ-chain cotransfection (8, 9).

FcγR bind IgG with different affinities and show class-specific binding to IgG subtypes. A defining characteristic of FcγRI is its high affinity for monomeric IgG, while FcγRII and FcγRIIIb bind primarily immune complexes, having low affinity for the monomeric ligand. The FcγRIIIa isoform binds monomeric IgG with an intermediate affinity that is lower than that of FcγRI but higher than that of FcγRIIIb and FcγRII (10). Although the high affinity binding of FcγRI for monomeric IgG (K_a = 10^{8-9} M^{-1}) (11, 12) has been attributed to the presence of the third extracellular domain (8, 13, 14), FcγRI transfected into COS cells binds ligand with an affinity that is fivefold lower (K_a = 5.0×10^7 M^{-1}) than that measured on cells that naturally express the receptor (8, 14). These results suggest that some factor(s), present in the cells that naturally express FcγRI and absent in COS cells, participates in the high affinity binding. Among these factors, one potential candidate is the γ-chain subunit. In the present study, we demonstrate that the affinity of both human and murine FcγRI increases when γ chain is cotransfected with these receptors, and that the affinity increase correlates with association with γ chain and is independent of the cytoplasmic domain of FcγRI. We also observed that the intermediate affinity of FcγRIIIa appears to be dependent upon γ-chain association.

Materials and Methods

Cells and Cell Culture. U937, a human monocyte cell line from American Type Culture Collection (Rockville, MD), and N10F7, a mouse 3T3 fibroblast cell line stably transfected with human FcγRI cDNA, were maintained in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), 2 mM

[1]*Abbreviations used in this paper:* FcR, Fc receptor; FcαR, IgA FcR; FcγR, IgG FcR; FcεR, IgE FcR; GPI, glycosyl phosphatidylinositol; K_a, equilibrium association constant; MFI, mean fluorescence intensity.

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L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. COS-7 cells were cultured in DMEM with the supplements listed above. The N10F7 medium also contained 0.5 mg/ml G418 (Life Technologies). U937 cells were cultured with 100 U/ml human rIFNγ (Genentech, San Francisco, CA) 48 h before each experiment to increase FcγRI expression.

Plasmid Constructs and Transfections. The human FcγRI cDNA cloned into the pCDM vector was described by Ernst et al. (3). The human γ-chain cDNA in vector pSVL was a kind gift from Dr. Jean-Pierre Kinet (National Institute of Allergy and Infectious Diseases, Bethesda, MD). FcγRIIIb, FcγRIIIa, FcγRIIIa GPI, and murine γ-chain cDNAs were all generous gifts from Dr. Jeffrey V. Ravetch (Memorial Sloan-Kettering Cancer Center, New York). FcγRIIIa and FcγRIIIb cDNA were further subcloned in the vector pcDNA1 (Invitrogen, San Diego, CA). The murine FcγRI cDNA was a kind gift from Dr. Duane W. Sears (University of California, Santa Barbara, CA), the human macrophage mannose receptor cDNA in the vector pHMR was from Dr. Benjamin A. Kruskal (Children's Hospital, Boston, MA), and the pRSVCAT vector was from Dr. Lai-Chu Wu (The Ohio State University, Columbus, OH). A truncated human FcγRI, designated FF, was constructed by PCR amplification using FcγRI cDNA as a template, and truncation was confirmed by sequencing. The FcγRI sense primer, containing a stop codon at the predicted amino acid residue 319, was 5'-GAC AAT ACG TAA AGA ACT GAA ATG AAA GAA-3', and the antisense primer was 5'-ACT GAG CCG CTG CTA CGT GG-3'. The cDNAs were transiently transfected into COS-7 cells by the diethylaminoethyl-dextran method described previously (3), using 1–2 μg/ml DNA, and the cells were analyzed 48 h after transfection.

Ligand-binding Assays and Scatchard Analysis. Approximately 5×10^5 COS-7 cell transfectants or N10F7 cells per well were cultured in 24-well plates overnight to confluence. Cells were incubated in triplicate for 2–4 h on ice with 0.15 ml of increasing concentrations (0.5–10 or 30 μg/ml) of monomeric ^{125}I-IgG2a labeled with an efficiency of 95% by the chloroglycouril method. After the cells were washed to remove unbound ^{125}I-IgG, they were solubilized in 0.5 ml 3 N NaOH, and radioactivity was measured with a gamma scintillometer. For cells in suspension, unbound ^{125}I-IgG was removed by centrifuging the cell suspension through oil (bis(2-ethylhexyl)phthalate/dibutylphthalate 1:1.1), as previously described (11). The nonspecific binding was determined by the amount of radioactivity binding to wells containing mock-transfected COS-7 and untransfected 3T3, or by incubating the COS-7, N10F7 transfectants, and U937 with radioligand in the presence of 100-fold excess unlabeled IgG2a. Both methods of determining nonspecific binding resulted in background counts that represented 0.1–0.3% of the input of radioactivity. Binding data were analyzed using the Collection of Radioligand Binding Analysis Programs (Elsevier-BIOSOFT, Cambridge, UK). Results were expressed as the mean ± SEM of 3–13 experiments, and statistical analysis was performed using Student's *t* test.

Immunoadsorption and Western Analysis. Anti-FcγRI mAb 197 (mIgG2a) was obtained from Medarex (Annandale, NJ). The rabbit anti–γ-chain serum was a generous gift from Dr. Jean-Pierre Kinet (National Institute of Allergy and Infectious Diseases), as was the mAb anti–γ-chain 4D8 from Dr. Jarema Kochan (Hoffman-La Roche, Nutley, NJ). COS-7 transfectants were lysed for 1–2 h on ice in 1% digitonin (Calbiochem, San Diego, CA), 20 mM triethanolamine, pH 7.8, 150 mM NaCl, 0.12% Triton X-100, and 2 mM PMSF (Sigma Immunochemicals, St. Louis, MO), and clarified supernatants were incubated with mAbs bound to Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) coupled to goat $F(ab')_2$ anti–mouse IgG (Pierce Chemical Co., Rockford, IL), as previously described (3). Immunoadsorbed proteins, eluted by boiling in Laemmli sample buffer containing 5% 2-mercaptoethanol, were separated by SDS-PAGE in parallel with Rainbow protein molecular weight markers (Amersham Corp., Arlington Heights, IL) and transferred to nitrocellulose membranes. After blocking, the blots were incubated sequentially with 1/1,000 dilution of anti–γ-chain rabbit serum and horseradish peroxidase–linked protein G (Bio Rad Laboratories, Hercules, CA) followed by enhanced chemiluminescence detection (ECL; Amersham).

Flow Cytometry Analysis. 2×10^5 COS-7 cells that had been transfected 48 h earlier with cDNAs for FcγRIIIa and γ chain, FcγRIIIb, or the FcγRIIIa GPI mutant were sequentially incubated with anti-FcγRIII mAb 3G8 and with FITC-labeled $F(ab')_2$ fragments of goat anti–mouse IgG, and were analyzed on an Elite EPICS Profile flow cytometer (Coulter, Hialeah, FL). Fluorescence data from 10,000 cells were expressed as the number of positive cells (percentage of the cells brighter than the mock-transfected COS-7 cells) and as the mean fluorescence intensity (MFI) of the positive cells.

Results

The Affinity for Ligand of Both the Human and the Murine FcγRI Increased when the Receptors were Cotransfected with the γ-Chain Subunit in COS Cells. Both the human and the murine FcγRI cDNAs were transfected into COS cells either alone or with γ chain, and the affinity of FcγRI for IgG was measured in ligand-binding assays using ^{125}I-labeled IgG2a, a murine IgG isotype that both species of FcγRI bind with high affinity (11). The saturation binding data, corrected for nonspecific binding and analyzed by the Scatchard method, produced a straight line for both the human FcγRI and murine FcγRI COS cells transfected with and without γ chain, which is indicative of a single population of receptors with a single affinity for IgG (Fig. 1). The equilibrium association constants (K_a) of the FcγRI transfectants were determined from the slope of the line obtained in the Scatchard plots (Table 1). The human and the murine FcγRI transfected into COS cells bound monomeric IgG2a with a K_a of $3.2 \pm 0.3 \times 10^7$ M^{-1} and a K_a of $4.9 \pm 0.4 \times 10^7$ M^{-1}, respectively (Table 1), similar to affinities previously reported for these transfectants (8, 14). The affinity for the ligand of FcγRI expressed in another fibroblast cell line, a murine 3T3 cell line stably transfected with a human FcγRI cDNA, was found to be nearly identical, with a K_a of 3.5×10^7 M^{-1}.

When the human γ chain was cotransfected with human FcγRI in COS cells, the binding affinity of the receptor for monomeric IgG2a was increased fivefold to a K_a of 15.6×10^7 M^{-1} (Fig. 1 and Table 1). The binding affinity of FcγRI expressed on IFN-γ–treated U937 cells, a human monocytic cell line that naturally expresses both FcγRI and γ chain, was also measured and an average K_a of 19.0×10^7 M^{-1} was found, which was in agreement with previous reports (11) and very similar to the binding affinity measured on the COS cells cotransfected with the human FcγRI and γ chain.

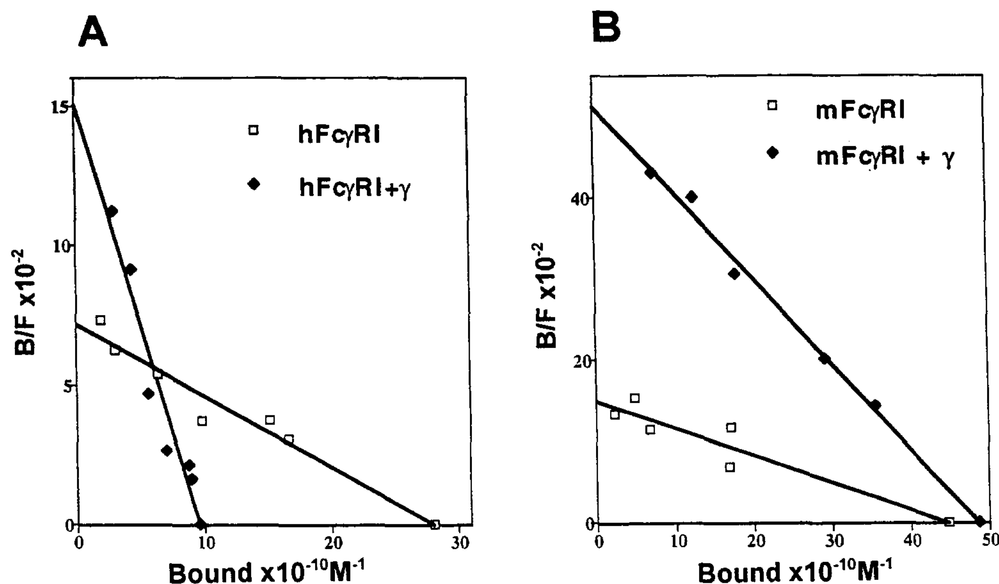

Figure 1. Scatchard plots of monomeric IgG binding to human and murine FcγRI transfected with and without γ chain in COS-7 cells. The Scatchard plot of one representative ^{125}I-IgG2a ligand binding assay is shown in which all points were done in triplicate and corrected for nonspecific binding, demonstrating the binding of ligand by FcγRI transfected alone (□) or with γ chain (◆). (*A*) Human FcγRI (*hFcγRI*) with and without human γ chain (*hγ*). (*B*) Murine FcγRI (*mFcγRI*) with and without murine γ chain (*mγ*). *B*, bound, molar concentrations of ^{125}I-IgG2a bound specifically to transfectants; *F*, free, molar concentration of unbound ^{125}I-IgG2a.

Table 1. *Equilibrium Association Constants for Binding of Monomeric IgG to FcγRI with and without γ Chain*

Cell type	Transfected cDNA	Affinity for ^{125}I-IgG2a: Human K_a (10^7 M^{-1})	*n*	Affinity for ^{125}I-IgG2a: Murine K_a (10^7 M^{-1})	*n*
U937		19.0	2		
P388DI				11.0‡	
N10F7	FcγRI	3.5	2		
COS-7	FcγRI	3.2 ± 0.3	13	4.9 ± 0.4	6
	FcγRI + hγ	15.6 ± 1.4*	10	9.2	2
	FcγRI + mγ	14.5	2	9.1 ± 0.3*	5
	FcγRI + control	3.2 ± 0.3	9	4.1 ± 0.7	3
	FF	6.9 ± 0.8	3		
	FF + hγ	20.3 ± 4.3*	3		

The results are tabulated from ligand-binding assays and Scatchard analyses of the binding of monomeric ^{125}I-IG2a to various cell types and COS-7 cells transfected with human or murine FcγRI cDNAs or a truncated form of FcγRI lacking the cytoplasmic domain (*FF*), with or without human or murine γ chain (*hγ*, *mγ*) cotransfection. Control cDNAs (*control*) cotransfected with FcγRI were the human mannose receptor cDNA and several expression vectors. K_a represent the mean of *n* ligand-binding assays ± SEM.

*The differences in the affinities of FcγRI for IgG that were measured when the receptor was transfected with or without the γ chain were statistically significant, as determined by the Student's two-tailed *t* test, with $P < 0.001$ for hFcγRI and ≤ 0.001 for mFcγRI.

‡The affinity of the mFcγRI expressed on the murine macrophage cell line P388DI, as reported by Unkeless et al. (12).

Similar results were obtained with the murine FcγRI cotransfected with γ chain in COS cells. Cotransfection of the murine γ chain induced a twofold increase in the affinity of the receptor for mIgG2a, with a K_a of 9.1 ± 0.3 × 10^7 M^{-1}, an affinity that is comparable to the one reported on the murine macrophage cell line P388DI, which had a K_a of 11.0 × 10^7 M^{-1} (12). The difference in binding affinities for both the human and the murine FcγRI that was measured when the receptor was transfected alone or with γ chain in COS cells was demonstrated to be statistically significant when analyzed by Student's two-tailed *t* test.

The human and the murine γ chains, which share 86% amino acid identity (15), substituted equally for each other in increasing the binding affinity of FcγRI. The human FcγRI cotransfected with the murine γ chain showed a K_a of 14.5 × 10^7 M^{-1}, which is almost identical to the affinity that was measured when cotransfected with the human γ chain. The binding affinity of the murine FcγRI, when cotransfected with the human γ chain, was also nearly identical to what was measured when the receptor was cotransfected with the murine γ chain, a K_a of 9.2 × 10^7 M^{-1}.

To exclude the possibility that the increase in binding affinity of FcγRI for ligand was a nonspecific effect of cotransfection, both the human and the murine FcγRI were cotransfected in COS cells with several control cDNAs, including the human macrophage mannose receptor cDNA and several expression vectors, pHMR, pRSVCAT, and pcDNAI. The binding of these FcγRI transfectants was analyzed as before, and the resulting K_a determined by the Scatchard method were 3.2 ± 0.3 × 10^7 M^{-1} and 4.1 ± 0.7 × 10^7 M^{-1} for human and murine FcγRI, respectively, when cotransfected with nonspecific cDNA (Table 1). Thus, cotransfection of nonspecific cDNA did not change the affinities of human and murine FcγRI.

Cotransfection of the γ chain with human FcγRI also appeared to affect the number of expressed receptors.

Binding of monomeric IgG2a to COS cells cotransfected with human FcγRI and γ chain showed a consistent decrease in the total concentration of receptor sites, as shown by the B_{max} values: $5.0 \pm 1.0 \times 10^{-10}$ M for FcγRI cotransfected with γ chain, as compared to $16.0 \pm 4.0 \times 10^{-10}$ M when FcγRI was transfected alone, or $13.0 \pm 1.0 \times 10^{-10}$ M when it was cotransfected with control cDNAs. Although the expression levels in transient transfectants can be variable, the consistency with which γ chain decreased the B_{max} of the human FcγRI suggested that the change in the concentration of receptor sites may be specific to γ-chain cotransfection. Unlike the human receptor, however, the B_{max} measured for the murine FcγRI cotransfected with γ chain was not different from the B_{max} obtained with FcγRI alone.

The Changes in Binding Affinity for IgG Correlate with the Association of γ Chain with FcγRI. We have previously shown that the association of the human FcγRI with human γ chain can be reconstituted in COS cells (3). To confirm that this association was present in our experimental conditions and to determine if the murine FcγRI association with the murine γ chain was also reconstituted in COS cell transfections, we analyzed transfectants by Western blot after immunoadsorption with antibodies against human FcγRI (197) or γ chain (4D8) (Fig. 2). Since antibody 197 is an IgG2a isotype, it was also used as a ligand to adsorb the murine FcγRI because of the lack of specific antibodies for this receptor. The anti–γ-chain immunoblot antibody recognized a 10-kD protein copurifying with FcγRI in immunoadsorbates from cells transfected with human FcγRI and human γ chain (Fig. 2 *A*, lane *2*), and from cells transfected with murine FcγRI and human γ chain (Fig. 2 *A*, lane *4*). A band that was the appropriate size for γ chain was also copurified from cells in which the human FcγRI was cotransfected with the murine γ chain (Fig. 2 *B*, lane *4*), as well as from cells cotransfected with the murine FcγRI and the murine γ chain (Fig. 2 *B*, lane *5*). Direct immunoadsorption of γ chain with anti–γ-chain mAb 4D8 confirmed the presence of γ chain in the appropriate cells (Fig. 2 *A*, lanes *2* and *4*, and *B*, lanes *4* and *5*).

The Association of FcγRI with γ Chain and the Subsequent Increase in Binding Affinity are Independent of the Cytoplasmic Domain of the Receptor. To investigate if the transmembrane domain of FcγRI was sufficient for association with γ chain as well as for the increase in binding affinity mediated by γ chain, a truncated form of the human FcγRI containing the extracellular and transmembrane domains of the receptor without the cytoplasmic domain (FF) was constructed and transfected into COS cells with or without γ chain. Western blot analysis with an anti–γ-chain antibody showed a 10-kD band (Fig. 2 *A*, lane *7*) in cells that had been cotransfected with the truncated construct and γ chain, and immunoadsorbed with either anti-FcγRI or anti–γ-chain antibodies, indicating that γ chain was expressed in these cells and was coimmunoadsorbed with the truncated FcγRI mutant.

The increase in binding affinity of FcγRI induced by γ chain was not affected by the absence of the cytoplasmic domain of the receptor. COS cells transfected with the truncated FcγRI alone bound monomeric ^{125}I-IgG2a with a K_a of $6.9 \pm 0.8 \times 10^{7}$ M^{-1}, whereas after cotransfection with γ chain, cells bound ^{125}I-IgG with a K_a of $20.3 \pm 4.3 \times 10^{7}$ M^{-1}, similar to the K_a of 19.0×10^{7} M^{-1} that was measured for FcγRI on U937 cells (Table 1), indicating that the cytoplasmic domain of FcγRI is not required for enhanced binding affinity. The binding affinity of the truncated FcγRI transfected without γ chain was twofold higher than that of the wild-type receptor ($P = 0.025$); however, no significant difference was seen in the affinity of the two receptors that were cotransfected with γ chain ($P = 0.400$).

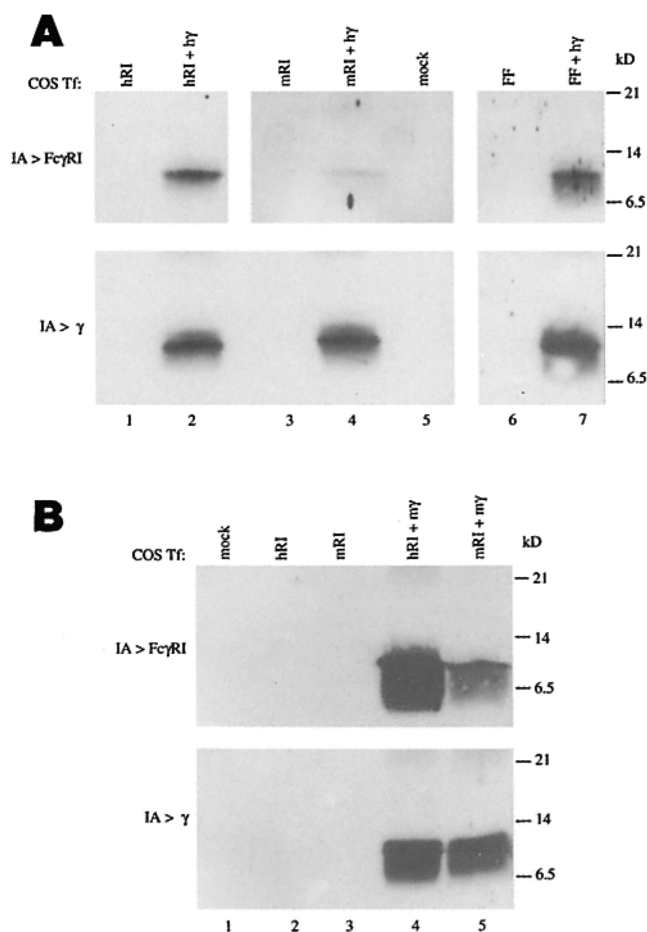

Figure 2. FcγRI association with γ chain in COS-7 transfectants. Digitonin lysates of COS-7 cells transfected (*COS Tf*) with human FcγRI (*hRI*) or murine FcγRI (*mRI*) with or without the human γ chain (*hγ*) (*A*, lanes *1–5*), and with or without the murine γ chain (*mγ*) (*B*, lanes *1–5*) were immunoadsorbed (*IA*) with anti-hFcγRI (*>FcγRI*, mAb 197; *A*, one-half the volume of the lysates; *B*, two-thirds the volume of the lysates) and anti–γ-chain mAb 4D8 (>γ). Lanes *6* and *7* in *A* are from a separate experiment that shows the association of γ chain with a truncated human FcγRI mutant lacking its cytoplasmic domain (FF) transfected with or without the human γ chain. The eluates were analyzed by Western blot with rabbit anti–γ-chain antibody and ECL detection. Lanes *3*, *4*, and *5* in *A*, >FcγRI are from a longer exposure time. Molecular masses of protein standards are indicated in kilodaltons.

Association with γ Chain Confers Medium Affinity Binding to FcγRIII. To determine if the medium affinity of FcγRIIIa

for monomeric IgG is caused by its association with γ chain, COS cells were transfected with either the FcγRIIIa isoform, which requires cotransfection and association with γ chain for cell-surface expression, or the FcγRIIIb isoform, a GPI-linked form of the receptor that does not associate with γ chain. We also transfected an FcγRIIIa mutant, resulting in a GPI-linked form of the receptor whose expression was independent of γ-chain association. The three FcγRIII constructs transfected in COS cells were analyzed simultaneously for ligand affinity by IgG-binding assay and for expression by flow cytometry. Table 2 shows the results of representative Scatchard and flow cytometric analyses of the same set of transfected COS cells, comparing ligand binding affinity and surface expression of the three FcγRIII. The affinity of FcγRIIIa cotransfected with γ chain (1.2×10^7 M^{-1}) demonstrated an ~10-fold increase when compared to the affinity measured for the FcγRIIIa GPI mutant (0.1×10^7 M^{-1}), while the affinity of FcγRIIIb could not be calculated because of the low level of ligand binding. The expression of the three FcγRIIIs was analyzed with an anti-FcγRIII antibody by flow cytometry to insure that the lower binding and affinities that were measured for FcγRIIIb and FcγRIIIa GPI were not caused by low levels of expression (Table 2). The three FcγR were expressed in COS cells, with FcγRIIIa/γ chain expressing less than the FcγRIIIb or FcγRIIIa-GPI mutant, confirming an absence of correlation between binding affinity and levels of receptor expression. Similar results were obtained in two additional ligand-binding assays and flow cytometric analyses.

Discussion

The functions that the γ chain provides in its association with FcγR continue to be defined. We have now shown that γ chain, in addition to its roles in receptor expression and signal transduction, modifies the affinity of FcγR for ligand. In this study, we demonstrated that the high affinity for monomeric IgG that characterizes both the human and murine FcγRI was dependent upon γ-chain cotransfection with the receptor, thus explaining the discrepancy between the lower binding affinity reported for FcγRI transfected into COS cells and the higher affinity measured on cells that naturally express the receptor. Association of γ chain increased the binding affinity of human FcγRI fivefold, and the affinity of the murine receptor twofold. While the level of affinity increase was lower in the mouse, the γ chain brought the affinity of both species of FcγRI to the levels reported on cells that naturally express the receptors. The differences observed between the human and the murine FcγRI when cotransfected with γ chain in COS cells did not appear to be related to the species of γ chain that was cotransfected with the receptors, since similar results were obtained when each receptor was cotransfected with the γ chain from the other species, suggesting differences within the structure of the receptors themselves. In fact, the reported range of binding affinity for the human FcγRI, K_a of 10^{8-9} M^{-1} (10), is slightly higher than the affinity reported on murine macrophages, K_a of 1.1×10^8 M^{-1} (12), an observation our data supports. Our findings also suggest that the FcγRIIIa isoform, the "medium affinity" FcγR, has a higher affinity for monomeric IgG than the FcγRIIIb isoform because of its association with γ chain. The affinity of an FcγRIIIa GPI mutant, expressed without the requirement of the γ chain, was 10-fold less than the affinity measured for FcγRIIIa cotransfected with the γ chain.

We had previously postulated that γ chain associated with FcγRI through the transmembrane domain of the receptor due to homology of this region to the transmembrane domains of FcεRI and FcγRIIIa (3), both shown to associate with γ chain through their transmembrane domains (16). With only five putative extracellular amino acids (15), it is unlikely that γ chain associated with FcγRI or induced its change in affinity through an interaction at the extracellular level. Analysis of a truncated FcγRI lacking its cytoplasmic domain not only supported the hypothesis that γ chain associated with the transmembrane domain of the receptor, but also demonstrated that the cytoplasmic domain of the receptor was not required for the increased binding affinity conferred by association with γ chain. In fact, we observed a slight but significant increase in the affinity of the truncated FcγRI when compared to the wild-type FcγRI, which suggests that the cytoplasmic domain may actually have an inhibitory effect upon binding affinity. When the receptors were cotransfected with γ chain, however, there was no longer a significant difference in the binding affinity of the truncated FcγRI compared to the wild-type receptor, suggesting γ-chain association may overcome any inhibitory effect of the FcγRI cytoplasmic domain to confer the appropriate receptor structure for high affinity binding.

It has been inferred previously that the unique third extracellular domain of FcγRI is responsible for its high affinity binding of monomeric IgG (8, 14). In one study, a mu-

Table 2. *Analysis of Ligand Binding and Expression of FcγRIII Transfected in COS-7 Cells*

Transfectant	Binding affinity K_a (10^7 M^{-1})	Expression	
		% + cells	MFI
FcγRIIIa + γ	1.2	10.9	28.5
Fcγ RIIIb	<0.1	22.0	62.6
Fcγ RIIIa GPI mutant	0.1	22.1	56.3

Equilibrium association constants of one representative ligand-binding assay of monomeric ^{125}I-IgG2a to COS-7 cells transfected with FcγRIIIa and the γ chain, FcγRIIIb, and an FcγRIIIa GPI mutant, each point done in triplicate as described in Fig. 1. The surface expression of the three FcγRIII constructs from the same set of transfected COS-7 cells was analyzed in flow cytometry with the anti-FcγRIII mAb 3G8. The results are expressed as the mean of the percentage of cells brighter than negative controls (*% + cells*) and the MFI of the positive cells. Similar results were obtained in two additional ligand-binding assays and flow cytometric analyses.

rine FcγR chimera lacking the third extracellular domain and containing only the first two extracellular domains of FcγRI spliced to the transmembrane and cytoplasmic domain of FcγRII bound IgG with the same low affinity and binding specificity as FcγRII (13). While we have shown that γ chain association is required for maximum binding affinity, FcγRI expressed without the γ chain still bound IgG with an affinity higher than the other FcγR, indicating that the high affinity binding of this receptor may be a result of contributions from the extracellular domain along with the association to the γ-chain subunit. The role of the extracellular domain was also apparent in our FcγRIII experiments. Both of the two GPI-linked forms of FcγRIII, a and b, bound ligand with low affinity; however, the affinity of FcγRIIIa could be measured, while the binding of FcγRIIIb was too low for an accurate affinity to be determined, even though the two receptors were expressed at similar levels. These findings suggest that the six-amino acid differences between the extracellular domain of FcγRIIIa and FcγRIIIb (17), in addition to association with γ chain, contributed to the higher binding affinity of this receptor. For both FcγRI and FcγRIII, it appeared that the structure of the extracellular domains affects binding affinity, and that association with γ chain further increases the affinity for ligand, probably through a change of the quaternary structure of the receptors.

While increased binding affinity due to an associated subunit is a novel observation among FcγR, a similar phenomenon has been described for members of the cytokine receptor family. Like FcγR, hematopoietic cytokine receptors are characterized by pleiotropy and redundancy, and lacking a tyrosine signaling motif in their cytoplasmic domain, they associate with common subunits including gp130, KH97, and the IL receptor γ chain (18–22), which are responsible for mediating the receptor signal. Like FcγRI, these cytokine receptors when expressed alone bind ligand with relatively low affinity but are transformed to high affinity receptor complexes upon association with their subunits. An explanation has been proposed for the affinity increase of these receptors upon association with the signaling subunit: receptors that do not have built-in signaling motifs and instead rely upon associated subunits might require a higher binding affinity for ligand to give the receptor the time that is necessary to transmit signals through the associated subunit (18). This hypothesis may also be relevant to FcγR, since FcγRII, which contains a signaling motif within its cytoplasmic domain, binds monomeric IgG with lower affinity than FcγRI and FcγRIIIa, both of which rely on association with the γ chain to transmit signals to the cell. It would appear that the ability of the γ chain to modify the affinity of FcγR for ligand is not only a novel observation among FcR, but it may also have a wider significance, suggesting that in addition to their roles in signal transduction, associated subunits may regulate receptor–ligand interaction.

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