The Cytoplasmic Domain of Human FcγRIa Alters the Functional Properties of the FcγRI-γ-Chain Receptor Complex*

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The γc-chain family of proteins mediate cell activation for multiple immunoglobulin receptors. However, the recognition that these receptors may have distinct biologic functions suggests that additional signaling elements may contribute to functional diversity. We hypothesized that the cytoplasmic domain (CY) of the ligand binding α-chain alters the biological properties of the receptor complex. Using macrophage FcγRIa as a model system, we created stable transfectants expressing a full-length or a CY deletion mutant of human FcγRIa. Both receptors functionally associate with the endogenous murine γ-chain. However, we have established that the CY of FcγRIa directly contributes to the functional properties of the receptor complex. Deletion of the CY of FcγRIa leads to slower kinetics of receptor-specific phagocytosis and endocytosis as well as lower total phagocytosis despite identical levels of receptor expression. Deletion of the CY also converts the phenotype of calcium independent FcγRIa-specific phagocytosis to a calcium-dependent phenotype. Finally, deletion of the CY abrogates FcγRI-specific secretion of interleukin-6 but does not affect production of interleukin-1β. These results demonstrate a functional role for the CY of FcγRIa and provide a general model for understanding how multiple receptors that utilize the γ-chain can generate diversity in function.

Fcγ receptors play a central role in the handling of immune complexes, regulation of inflammatory responses, antibody secretion, and T cell activity (1–4). Common to each of these functions is the initiation of tyrosine phosphorylation following receptor cross-linking (5) and the involvement of the γc subunits leading to the view that Fc receptors subserve redundant signaling functions. However, recent evidence suggests that these receptors are not redundant. For example, FcγRIIIa appears necessary for initiating the Arthus inflammatory reaction (6, 7), while FcγRI and FcαRI can down-regulate inflammatory responses by initiating the secretion of IL-10 and IL-1ra, respectively (4, 8). The basis for these differences are unknown.

FcγRI is expressed on the cell surface in association with the γ-chain (9, 10). This association is not a prerequisite for transient receptor expression but is necessary for stable expression (11, 12). The γ-chain cytoplasmic domain contains an immunoreceptor tyrosine activation motif (ITAM) and current data suggest that the γ-chain cytoplasmic domain is both necessary and sufficient for FcγRIa induced functions (13–15). Biochemical studies have shown that cross-linking of the FcγRI-γ-chain complex results in activation of a Src family kinase(s) and the tyrosine kinase p72Syk (2, 5). Activation of these kinases results in tyrosine phosphorylation of the γ-chain and the initiation of a signaling cascade that can culminate in the induction of granulocyte/macrophage colony-stimulating factor, phagocytosis, an oxidative burst, adhesion molecule and the induction of gene transcription. The association between FcγRIa and γ-chain may also be important in the formation of a higher affinity receptor complex through the recruitment of two ligand binding chains to the γ homodimer (16).

Unlike the γ-chain, the cytoplasmic domain of FcγRI does not contain an ITAM or other tyrosine containing signaling motifs. Nonetheless, murine FcγRI on J774 cells is constitutively phosphorylated on serine and after phorbol 12-myristate 13-acetate stimulation the level of phosphorylation increases (17). The cytoplasmic domain of FcγRI may also associate with actin-binding protein-280 (ABP-280, also known as non-muscle filamin) in the absence of ligand (18). Receptor engagement by ligand apparently abrogates this association, although its functional significance is not clear. Both of these observations suggest that the cytoplasmic domain of FcγRIa may be actively involved in the biologic phenotype of FcγRI. Furthermore, FcγRIa in the absence of the γ-chain can signal for calcium in COS-1 cells and the transmission of this calcium signal requires the FcγRIa cytoplasmic domain (19). Based on these observations, and the observations that several γ-chain associated Fc receptors initiate functionally distinct cell programs, we hypothesized that the FcγRIa cytoplasmic domain may serve to modify the signaling of the FcγRI-γ-chain receptor complex. By directly comparing wild type human FcγRIa with a cytoplasmic domain deletion mutant of FcγRIa expressed at comparable levels in stable transfectants of the murine macrophage cell line P388D1, we have established that the cytoplasmic domain of human FcγRIa alters the functional properties of the FcγRI-γ-chain receptor complex.

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mic domain of FcγRIα alters the functional properties of the receptor complex. These observations provide a general framework for understanding the unique properties of the family of Fcγ receptors which associate with the γ-chain.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents—**The murine macrophage cell line P388D1 stably transfected with a cDNA encoding human FcγRIα or a mutant form of FcγRIα containing a stop codon after the first amino acid of the cytoplasmic domain (Lyse+/+—Stop 315) were described previously (19). P388D1 cells, with human FcγRIα were previously described (20). All cell lines were maintained as adherent cultures (Corning Tissue Culture Dishes) in RPMI 1640 as described previously (20). All tissue culture reagents were from Life Technologies, Inc. (Grand Island, NY).

Human and mouse IgG were obtained from Sigma. Mouse Fab(′)2 fragments and F(ab′)2 goat anti-mouse IgG (GαM) were obtained from Jackson ImmunoResearch (West Grove, PA). Fab(′)2 fragments of the anti-FcγRI mAbs 22.2 and 22.2 were obtained from Medarex (Annandale, NJ). IgM anti-H2-D9*(clone 3-25-4) was obtained from Pharmingen (San Diego, CA). The hybridoma line expressing the rat anti-murine FcRII/RIIFcγRII mAb 2.4G2 was obtained from ATCC (Manassas, VA). All other reagents were from Sigma. Quantitative huFcγRI expression was assessed by resting the cell expressing the wild type (WT) and the cytoplasmic domain deletion mutant (MTD) by flow fluorescent activated cell sorting using anti-FcγRI mAb 22.2-FITC (Medarex).

A polyclonal anti-γ-chain Ab (666) was kindly provided by Dr. Jean-Pierre Kinet (21). In addition, polyclonal anti-γ-chain Abs were prepared in rabbits immunized with a C-terminal peptide sequence that is shared by both human and murine γ-chain exactly as described (21). To verify the specificity of the polyclonal antibodies, γ-chain from U937 cells was immunoprecipitated with protein G-agarose bound anti-γ-chain mAb (mAb 4D8) (kindly provided by Dr. J. Kochan (22) followed by immunoblotting with the polyclonal Abs (see below).

**Immunoprecipitation and Phosphotyrosine Analysis—**FcγRI was immunoprecipitated from the transfected lines using either mAb 22.2 or Fab′1,9-galactosidase (Amerham Pharmacia Biotech, Piscataway, NJ). γ-Chain from transfected cells was immunoprecipitated by polyclonal rabbit anti-γ-chain Abs bound to protein G-agarose (or from U937 cells with protein G-bound mAb 22.2). Cells (10–20 × 10⁶/ml) were lysed in PBS containing either 1% Nonidet P-40 (Sigma) or 1% digitonin (Wako Biochemicals, Waco, TX) and incubated in the presence of proteinase inhibitors. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting.

**For immunoblotting analysis, immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes (24). Membranes were blocked with 10% non-fat milk fol lowed by incubation with either polyclonal anti-γ-chain Ab or anti-phosphotyrosine (4G10 (UBI)) or biotinylated F(ab′)2 or F(ab′)2 fragments of the absorbed proteins were washed three times with PBS, 0.1% Tween 20 and bound mAb or Ab was detected with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (Amersham Pharmacia Biotech or Jackson ImmunoResearch). Following 3 more washes, bound Ab was detected using ECL (Amersham Pharmacia Biotech) according to the manufacturer’s directions. Membranes were stripped by incubation with Tris-HCl, pH 2.3, for 30 min at room temperature and then re-probed as described above.

**Analysis of [Ca²⁺]i—**Fura-2 (Molecular Probes, Eugene, OR), a fluorescent dye with spectral properties that change with the binding of free Ca²⁺, was used to measure changes in intracellular calcium concentrations as we have described (25). P388D1 cells, adhered to 25-mm diameter round glass coverslips at 5 × 10⁵ cells/ml, were incubated at 37 ºC for 15 min with 2 μM fura-2 AM. The resulting populations were washed 3 times with PBS, 0.1% Tween 20 and bound mAb or Ab was detected with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (Amersham Pharmacia Biotech or Jackson ImmunoResearch). Following 3 more washes, bound Ab was detected using ECL (Amersham Pharmacia Biotech) according to the manufacturer’s directions. Membranes were stripped by incubation with Tris-HCl, pH 2.3, for 30 min at room temperature and then re-probed as described above.

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**Cytokine Analysis—**Cells were stimulated in 96-well tissue culture plates (Corning) with phorbol 12-myristate 13-acetate, surface absorbed rabbit IgG, or surface absorbed Fab′2 goat IgG mAb 22.2 or Fab′2 goat anti-mouse IgG (Jackson Immunosorbent assay. For IL-1β detection by flow cytometry, recombinant IL-1β capture ab (polycrystal rab Ab), and biotinylated detection and neutralization mAb (clone 1400.24.17) were obtained from Endogen (Woburn, MA). For IL-6 determination, recombinant standard, capture mAb (clone MP5-2-F3), and biotinylated detection mAb (clone MP5-32CL1) were obtained from Pharmingen. Horseradish peroxidase-conjugated streptavidin (Jackson) and then TMB substrate were added and the absorbance at 490 nm was determined.

**Flow Cytometry—**Aliquots of cells at 5 × 10⁵ cells/ml were incubated with saturating concentrations of primary mAb for 30 min at 4 ºC followed by two washes. For indirect immunofluorescence, the cells were then incubated with saturating concentrations of FITC-conjugated goat anti-mouse IgG (Pharmingen) for 30 min at 4 ºC. After washing, the cells were analyzed immediately for immunofluorescence using a FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA).

**Statistical Analysis—**Analysis of flow cytometry listmode data was done using CellQuest (Becton Dickinson Immunocytometry). Statistical comparisons were performed with the paired t test. A probability of 0.05 was used to reject the null hypothesis that there is no difference between the samples.
clones with identical levels of receptor expression (Fig. 1) that were studied. Transfected cell lines were sorted to generate a saturating concentration of the anti-human Fc face of stably transfected P388D1 cells. Cells were incubated with the Fc of Fc (18). Furthermore, Fc g g endogenously expressed murine Fc g arylated on serine and/or threonine residues (17) and human Fc g RI receptor complex (9, 10). The transmembrane regions of Fc gRIa and the -chain mediate receptor complex assembly and 20 of the 21 amino acids in the transmembrane region are identical in murine and human g-chain with one conservative difference (I → V). As predicted, comparable amounts of -chain was co-immunoprecipitated from the WT and MUT lines after cell lysis in buffer containing 1% digitonin (Fig. 2). As a positive control, we co-immunoprecipitated -chain with endogenously expressed murine Fc gRII and Fc gRIIa using mAb 2.4G2. In accordance with the -chain association data, cross-linking of the transfected WT and MUT huFcRIa with mAb 22.2 Fab(1′)2 + Fab(2′)2 GaM resulted in tyrosine phosphorylation of the -chain (results not shown) demonstrating that both WT and MUT huFcRIa associate with the endogenous murine g-chain and initiate tyrosine phosphorylation of the -chain.

The huFcgRIa CY Domain Alters the Magnitude and Kinetics of Fc gRIa Internalization—While devoid of tyrosine residues, the -chain of murine Fc gRIa has been shown to be phosphorylated on serine and/or threonine residues (17) and human Fc gRIa has been shown to bind to ABP under some conditions (18). Furthermore, Fc gRIa in the absence of the -chain signal for calcium in COS-1 cells and the transmission of this calcium signal requires the Fc gRIa cytoplasmic domain (19). Accordingly, we considered the possibility that the CY domain of huFcRIa may contribute to the functional properties of the receptor complex. Using erythrocytes coated with the anti-human Fc gRIa mAb 22.2 Fab(1′)2, both WT and MUT huFcRIa mediated receptor-specific phagocytosis (Fig. 3). However, the WT construct consistently displayed a higher phagocytic index despite identical levels of receptor expression (Fig. 3). There was no internalization of E-22.2 by parental non-transfected P388D1 cells and no phagocytosis of erythrocytes coated with an IgM anti-H-2Dd mAb (clone 3-25.4) by any cell type (Fig. 3A), despite comparable binding of the E-3-25.4 probe to the transfected cells when compared with E-22.2.

We considered the possibility that engagement of the ligand-binding site of Fc gRIa, which can augment receptor function (28), might alter the difference in phagocytic capacity between the WT and MUT Fc gRI. However, saturation of Fc gRIa with mlgG2a did not alter the magnitude of Fc gRI-specific phagocytosis and did not abrogate the quantitative difference in phagocytosis between WT and MUT Fc gRIa (Table I).

Phagocytosis by WT huFcgRIa also displayed more rapid kinetics (Fig. 3B). Similarly, while both WT and MUT huFcgRIa were capable of endocytosis, endocytosis by the WT receptor was more rapid than that mediated by the MUT receptor (Fig. 4). Endocytosis of endogenous murine Fc gRIa, assessed on non-transfected P388D1 cells, was indistinguishable from the transfected huFcgRIa. Since we have matched the WT and MUT cell lines for receptor expression, the differences in phagocytic capacity and the more rapid kinetics of phagocytosis and endocytosis by WT Fc gRI provide the first evidence that the cytoplasmic domain of Fc gRIa, in association with the -chain, can affect receptor function.

The CY Domain of the -Chain Determines Ca2+ Sensitivity of Fc gRIa Phagocytosis—Through the use of chimeric and mutant receptors, the ITAM has been shown to be both necessary and sufficient for Fc gRI phagocytosis and the Fc gRI Ca2+ transient (14, 15, 20, 29–31). The functional importance of the Ca2+ transient has been demonstrated with huFcgRIa which incorporates an ITAM directly in the CY domain and requires elevations in intracellular Ca2+ to mediate phagocytosis (20). In contrast, Fc gRIa/-chain specific phagocytosis is independent of the receptor-induced Ca2+ transient (20), and we considered the possibility that the CY domain of Fc gRIa confers a Ca2+-independent phenotype on Fc gRIa-specific phagocytosis. When intracellular Ca2+ levels were quenched with BAPTA (resulting in [Ca2+]e = 57 ± 9.3 nM with 20 μM BAPTA treatment), receptor-specific phagocytosis induced by the WT huFcgRIa was unaltered (Fig. 5A), as we have previously shown for Fc gRI on human monocytes. In contrast, receptor-specific phagocytosis induced by the MUT huFcgRIa was blocked by pretreatment of the cells with BAPTA in a dose-dependent manner (Fig. 5A).

Since the absolute level of MUT huFcgRIa phagocytosis is lower than WT huFcgRIa, we considered the possibility that the BAPTA sensitivity might be related to the quantitative level of phagocytosis. Accordingly, phagocytosis by the WT huFcgRIa was performed with E-22.2 prepared with a lower mAb conjugation level resulting in a phagocytic index of 65.1 ± 13.2 compared with a phagocytic index of 70.4 ± 6.2 for MUT
FIG. 3. Receptor-specific phagocytosis by WT and MUT human FcγRIa. A, quantitation of human FcγRIa-specific phagocytosis (E:22.2 F(ab')2, hatched bars, n = 15) or H-2D^b-specific phagocytosis (E:3–25.4, solid bars, n = 3) by WT and MUT human FcγRIa stable transfectants. As a control, parental non-transfected cells were analyzed. Phagocytosis was performed as described under “Experimental Procedures” and quantitated by light microscopy. Data are expressed as the mean phagocytic index ± S.D. B, kinetics of phagocytosis of E:22 F(ab')2 by WT (○) and MUT (●) human FcγRIa P388D1 stable transfectants. Phagocytosis was performed as described under “Experimental Procedures” and quantitated by flow cytometry. Data are presented from a single representative experiment (n = 5). * p < 0.001, FcγRIa-specific phagocytosis by MUT versus WT.

TABLE I

| Phagocytic index | Control | +20 μg/ml mIgG2a |
|------------------|---------|------------------|
| WT hFcγRIa transfectants | 176.1 ± 41.6^a | 140.0 ± 13.8 |
| MUT hFcγRIa transfectants | 116 ± 37.3 | 128.2 ± 49.8 |

^a Data represent the mean ± S.D. (n = 7 for WT and n = 5 for MUT). p > 0.05 for control versus + mIgG2a for both WT and MUT.

FIG. 4. Receptor-specific endocytosis by WT and MUT human FcγRIa in P388D1 stable transfectants and of murine FcγRI in non-transfected P388D1 cells. Internalization of WT huFcγRIa (●), MUT human huFcγRIa (○), and murine FcγRI (△) after receptor-specific cross-linking was determined by flow cytometry as described under “Experimental Procedures.” Data are presented as the mean ± S.D. from a total of eight experiments. * p < 0.01 (1 min) and p < 0.005 (2 min and 5 min), WT versus MUT % internalized.

huFcγRIa and E:22.2 prepared at the maximal conjugation level. WT huFcγRIa and E:22.2 were prepared at the maximal conjugation level. WT huFcγRIa insensitivity to BAPTA was maintained under these reduced phagocytic conditions (Fig. 5A) indicating that the Ca\(^{2+}\) insensitivity is a property of the CY domain of huFcγRIa. As an additional control, FcγRIa-specific phagocytosis by P388D1 cells expressing full-length huFcγRIa (with a phagocytic index of 167 ± 32.6) was also shown to be blocked by pretreatment of the cells with BAPTA (Fig. 5B), as we have previously reported (20). Importantly, both WT and MUT huFcγRIa receptor complexes induced indistinguishable Ca\(^{2+}\) transients when cross-linked with anti-receptor mAb (results not shown). Thus, WT huFcγRIa engages a Ca\(^{2+}\)-insensitive phagocytic pathway while MUT huFcγRIa with the associated γ-chain engages a Ca\(^{2+}\)-sensitive phagocytic pathway. These results provide additional evidence that the CY domain of the ligand-binding α-chain of huFcγRIa alters functional properties of γ-chain ITAM-dependent functions.

Requirement of the FcγRIa γ-Chain for the Induction of IL-6 Secretion—In addition to its role in internalization, FcγRIa can also modulate the immune response through the induction of cytokine secretion. In particular, activation of monocytes/macrophages by FcγRIa can result in the secretion of IL-6 and IL-1β (32, 33). Accordingly, P388D1 expressing the WT and MUT forms of huFcγRIa were stimulated with receptor-specific mAb bound to surface absorbed F(ab')2-GaM. Quantitation of IL-1β secretion after cross-linking of huFcγRIa demonstrated that both WT and MUT forms of the receptor were capable of eliciting comparable levels of secretion of this cytokine (Fig. 6). Cells incubated in the presence of GaM alone were not stimulated to secrete IL-1β above the baseline control. In contrast, cross-linking WT huFcγRIa, but not MUT FcγRIa, induced the secretion of IL-6 (8-h time point). We did detect IL-6 production above baseline at 24 h after MUT FcγRIa stimulation; however, neutralization of endogenously produced IL-1β prevented this induction of IL-6 secretion by the MUT FcγRIa after 24 h culture (253 ± 52 pg/ml and 75 ± 21 pg/ml in the absence and presence of a neutralizing anti-IL-1β mAb). In contrast, neutralizing anti-IL-1β mAb did not abrogate the IL-6 induction observed at the 4- or 8-h time points by WT huFcγRIa. No significant difference in the ability of phorbol 12-myristate 13-acetate (100 ng/ml) or surface bound IgG, engaging endogenous murine FcγRIIa/FcγRIIia and transfected human FcγRIa, to elicit IL-1β or IL-6 secretion was observed between the WT and MUT lines (results not shown). These results document the requirement for the α-chain of the FcγRIa receptor complex for the induction of the IL-6 response by the receptor complex and demonstrate that the pathways leading to IL-6 secretion and IL-1β secretion are distinct.

DISCUSSION

Three lines of evidence indicate that the FcγRIa γ-chain complex induced responses are altered by the cytoplasmic domain of the α-chain. First, when matched for receptor expression, WT FcγRI has a higher phagocytic capacity than the cytoplasmic domain deletion mutant. The WT receptor complex also displays faster kinetics of phagocytosis and endocytosis despite comparable kinetics of tyrosine phosphorylation of the γ-chain. Second, the cytoplasmic domain of FcγRI confers a calcium-independent phenotype on phagocytosis by the receptor complex. Third, and most importantly, we have observed that the
induction of IL-6 secretion requires the cytoplasmic domain of the FcγRⅠa-α-chain (Fig. 6). Taken together, these data provide the first direct demonstration of a functional role of the cytoplasmic domain of FcγRI in the FcγRI-γ-chain complex.

Previous studies of FcγRI transiently transfected into COS cells have suggested that cross-linking of the α-chain may mediate changes in intracellular calcium and endocytosis (19, 34, 35). However, in the absence of γ-chain, FcγRIa is not competent for phagocytosis (13–15). The central role of the γ-chain and the tyrosine kinase p72Syk, which docks via SH2 domains to the tyrosine-phosphorylated γ-chain, has been demonstrated through a series of experiments using co-transfection of the FcγRIa-γ-chain complex with Syk kinase and transfection of chimeric receptor constructs incorporating either the γ-chain cytoplasmic domain or Syk kinase directly (36, 37). Furthermore, the observation that both 1) human monocytes/macrophages in which Syk has been specifically ablated by antisense constructs (38) and 2) murine macrophages from Syk knockout mice (39, 40) are incapable of Fcγ receptor-mediated phagocytosis supports the conclusion that the γ-chain induced activation of Syk is necessary and sufficient for a phagocytic response. Given these results, our observations that the cytoplasmic domain of FcγRIa modulates the kinetics of phagocytosis may reflect the association of cytoskeletal elements with the cytoplasmic domain and subsequent changes in the mobility of the receptor in the plasma membrane. Such a mechanism may explain the observation that FcγRI in the NOD mouse also shows altered kinetics of endocytosis (41).

The change in calcium independent signaling elements and in the induction of IL-6 synthesis indicates that the cytoplasmic domain of FcγRI also affects the nature of intracellular signals generated by the FcγRI-γ-chain receptor complex. Studies of the high affinity receptor complex for IgE comprised of ligand binding α-chain, a single β-chain, and a γ-chain homodimer indicate that the β-chain is constitutively associated with the Src kinase Lyn and can recruit protein kinase C-δ both of which can modulate the signaling capacity of the ITAM in the γ-chain (42–45). In this manner, the β-chain acts as an amplifying mechanism and, although the role of serine/threonine phosphorylation of γ-chain is not yet established, the presence of serine/threonine phosphorylation targets within the γ-chain provide an attractive target for regulation. Indeed, FcγRIa cross-linking on U937 cells results in the serine phosphorylation of the γ-chain (46) and recruitment of protein kinase C-β and protein kinase C-ε to the membrane by Fcγ receptors during phagocytosis has been documented (47). In a parallel fashion, perhaps, the cytoplasmic domain of FcγRIa may recruit signaling elements to the receptor complex. Although it contains no ITAM the FcγRIa cytoplasmic domain may directly transmit the signal for IL-6 release in the absence of participation of the γ-chain.

It is also interesting to note that subtle differences in the ITAM sequences used by Fc receptors may also contribute to distinct biological properties. The ITAM-like sequence in FcγRIa differs from the ITAM in the γ-chain and this difference, or other adjacent sequences, influence the relative dependence on intracellular calcium transients for phagocytosis (Fig. 5). This property and the differences in serine/threonine residues within the ITAM may allow for differences in some of the functions between FcγRIa and FcγRIb/FcγRIIBa.

The mechanism(s) by which the cytoplasmic domain of FcγRIa alters receptor function is not clear. Murine FcγRI is phosphorylated on serine/threonine residues after phorbol 12-myristate 13-acetate stimulation (17). However, the functional importance of this phosphorylation has not been examined. Differences in the association of WT and MUT FcγRIa with γ-chain cannot be the mechanism for the observed functional differences between these receptors. Transfectants have been sorted for identical cell surface receptor expression and both WT and MUT associate equally with γ-chain (by direct co-immunoprecipitation and functionally by receptor induced tyrosine phosphorylation). Recent studies have also demonstrated that association with γ-chain is essential for stable expression of huFcγRIa (11, 12). A direct interaction between
human FcγRα and ABP-280 (non-muscle filamin) has been reported (18). Although there are no known consequences of the interaction between FcγRα and ABP, ligand engagement of FcγRα results in both the dissociation of the receptor from ABP-280 and in the enhancement of the FcγRα triggered oxidative burst in U937 cells (28). In the present study, we have used an anti-human FcγRα mAb F(ab′)2 fragment that does not engage the ligand-binding site (23). In this transfection system, saturation of FcγRα not engage the ligand-binding site (23). In this transfection system, saturation of FcγRα not engage the ligand-binding site (23). In this transfection system, saturation of FcγRα not engage the ligand-binding site (23).

Demonstration that the cytoplasmic domain of the α-chain of FcγRα alters the functional properties of the FcγRI-γ-chain complex provides a mechanism for unique biologic properties initiated by each receptor complex. Given the diversity in primary sequence of the cytoplasmic domains of the γ-chain-associated Fc receptors, the opportunity for these unique domains to confer distinct functions on each receptor complex is clear. These observations also suggest that single nucleotide polymorphism leading to missence mutations in the cytoplasmic domains of these receptors may have biological significance. We have recently described two single nucleotide polymorphisms in the cytoplasmic domain of FcγRα proximate to putative phosphorylation sites (48). These polymorphisms may provide another level of functional variation which builds upon a general framework of the role of sequence variations in the cytoplasmic domains altering the functional properties of the receptor complex.

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