FCγ receptor γ chain has previously been shown to interact with the TCR-CD3 complex, the IgE Fc receptor I (FcεRI), and the class I and II myeloid Fc receptors (FcγRI and FcγRIIa). Here, we demonstrate that the Fc receptor γ chain associates with FcγRI in transfected IIA1.6 B lymphocytes. FcγRI could be expressed at the surface of IIA1.6 B cells by itself, but was devoid of signaling capacity. Upon co-expression of Fcγ γ chain, a physical interaction with FcγRI could be demonstrated. This association proved crucial for the triggering of both proximal (intracellular calcium increase and tyrosine phosphorylation), as well as distal (IL-2 release), signal transduction responses. We next tested the hypothesis that a positively charged arginine residue (Arg209) within the transmembrane domain of FcγRI promotes association with Fcγ γ chain. We therefore constructed FcγR molecules where Arg209 was mutated to either a positively charged histidine, a negatively charged aspartic acid, or an uncharged leucine. A functional association between FcγRI and Fcγ γ chain was observed only with a positively charged residue (Arg209 or His209) present within the FcγRI transmembrane domain. These data show that transmembrane signal transduction by the FcγR is mediated via Fcγ γ chain, and that FcγRI requires a positively charged residue within the transmembrane domain to promote functional association.

IgA is the primary immunoglobulin in bodily secretions and plays a critical role in protection against the constant environmental challenges at mucosal sites. Although the protective mechanisms are incompletely defined, a significant role in IgA-mediated immune defense has been proposed for IgA Fc receptors. These molecules have been detected on most populations of phagocytic cells in blood and mucosal tissues. Engagement of these molecules can trigger phagocytosis, degranulation, oxidative burst, inflammatory mediator release, and antibody-dependent cellular cytotoxicity (1, 2). FcγR on monocytes/macrophages and neutrophils has been defined as a 55–75-kDa glycoprotein (3, 4), whereas the eosinophil FcγR is more heavily glycosylated (70–100 kDa) (5). Both types of myeloid receptors are recognized by the CD89 mAb (4) and bind both IgA1 and IgA2 via their Fc regions (1). The cDNA encoding the myeloid FcγR has been characterized and was found to encode a 30-kDa peptide, with two extracellular Ig-like domains, a hydrophobic transmembrane region and a cytoplasmic tail devoid of recognized signaling motifs (2, 6). Additionally, we have recently isolated and characterized the human gene encoding the CD89 molecule. The gene structure indicates FcγR to represent a more distantly related member of the immunoglobulin receptor gene family (7). To explore the capacity of FcγR to trigger biological functions, we have now generated different transfectants in the mouse IIA1.6 B cell line. This line, derived from the A20 B cell lymphoma, lacks the 5′ end of the FcγRII gene and, consequently, is Fcγ receptor-negative (8). Previous work showed this line to represent an excellent model for assaying FcγR-mediated functions (9–11). Following transfection, FcγR was expressed at the surface of IIA1.6 cells by itself, but lacked signaling capacity. We therefore hypothesized that FcγR may associate with a specialized signaling molecule. The Fcγ γ chain was known previously to associate with all three classes of FcγR, FcεRI, and the TCR-CD3 complex (12–15). FcγR γ chain is responsible for coupling these receptors to intracellular signaling pathways (16). By co-transfection experiments, we tested whether Fcγ γ chain could mediate signal transduction via FcγR. Our results show that co-expression of FcγRI and Fcγ γ chain in IIA1.6 B cells conferred both proximal and distal signaling capacity to FcγR. During the preparation of this manuscript, it was shown that, in the U937 cell line, FcγR was associated with Fcγ γ chain and that γ chain was phosphorylated on tyrosine residues following FcγR cross-linking (17). The data presented here indeed confirm that FcγR associates with Fcγ γ chain in transfected IIA1.6 B cells and, furthermore, suggest that FcγRI and Fcγ γ chain can associate in normal blood PMN. The present experiments demonstrate Fcγ γ chain to be critical for FcγR-mediated transmembrane signal transduction.

We, furthermore, explored the molecular basis for FcγRI/FcγR γ chain association. The transmembrane (TM) domain of FcγRI is unusual as it contains a single positively charged arginine (Arg209) residue. Since the TM domain of the Fcγ γ chain...
contains a single negatively charged aspartic acid residue, we hypothesized that these oppositely charged residues may promote association. A similar mechanism involving charged TM amino acids has previously been shown to be involved in the assembly of the TCR-CD3 complex (18–21). Using PCR, we mutated the Arg209 found in the wild type FcR (R209D) to either a positively charged histidine (R209H), a negatively charged aspartic acid (R209D), or an uncharged leucine (R209L). Mutated FcR cDNAs were transfected together with FcRγ chain to IIA1.6 cells and assessed for their ability to trigger an increase in [Ca²⁺], following FcR cross-linking. Our data show a positively charged residue within the TM domain of FcR to be required for functional association with FcRγ chain.

MATERIALS AND METHODS

Cells—The murine B cell line IIA1.6 (8) and the human cell line U937 were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 1 mM sodium pyruvate. FcRγ and FcRγ/γ transfectants were maintained in the same medium supplemented with 1% heat-inactivated FCS (Gibco/BRL), Life Technologies, and either alone or Gentamicin and methotrexate (10 μM; Pharmachemie, Haarlem, The Netherlands), respectively. PMN were purified as described previously (22). Murine macrophages were isolated from the peritoneal cavity by rinsing with RPMI 1640, 10% fetal calf serum.

Expression Vectors and Transfections—The human FcRγ cDNA containing a promoter vector was a gift from Dr. J. C. Maliszewski (6). The murine FcRγ chain cDNA (23) was cloned into the pNUT expression vector (pNUT-γ), allowing selection of transfected cells with methotrexate (24). Transfection of IIA1.6 B cells was performed by electroporation as described previously (10). IIA1.6 B cells were stably transfected with FcR alone (FcRγ) or with FcRγ and chain (FcRγ/γ) as follows. FcRγ cells were generated by co-transfection of FcRγ cDNA (pCAG-GUV, using pRC-CMV neo as selection marker (10)). Two days following transfection, cells were transferred to medium containing G418 and seeded in 24-well plates. After 14 days, each well was tested for FcRγ expression by FACS analysis using anti-FcRγ antibody (9). For FcRγ/γ cells, the same procedure was used except that 25 ng of each overlapping DNA fragment was added as template along with 10 pmol of primers 5′-CGTGGCA-3′ and 1 unit of AmpliTaq DNA polymerase (Perkin Elmer) was used to amplify mutated cDNA fragments. PCR products were subcloned into pGEM-T (Promega) vectors, and the integrity of all mutants was confirmed by sequence analysis. Mutant cDNAs were cloned into the eukaryotic expression vector pSG5 (27) prior to transfection as described above.

Immunofluorescence—Cells were incubated with either mAb A77 or My34 culture supernatant for 30 min at 4°C. Cells were washed twice with PBS, 1% bovine serum albumin, 0.1% NaNO₃, and subsequently incubated with either FITC-conjugated goat anti-mouse (GAM) IgG1 or FITC-conjugated GAM IgM, respectively (Southern Biotechnology). Following incubation at 4°C, cells were washed twice and analyzed on a FACSscan flow cytometer (Becton Dickinson). For intracellular staining, cells were permeabilized by incubation in FACS Lysing Solution (Becton Dickinson) for 10 min at room temperature. After washing, FcRγ chain expression was detected by incubation with a rabbit anti-serum against FcRγ chain (generously provided by Dr. J.-P. Kinet; Ref. 28) followed by FITC-conjugated goat anti-rabbit F(ab′)₂ fragments (Southern Biotechnology).

Calcium Mobilization Assays—Intracellular free calcium levels were analyzed using a FACSscan (10). Briefly, cells were loaded simultaneously with SNARF-1 (2.8 μM) and Fluo-3 (1.4 μM) (Molecular Probes) by incubation for 30 min at 37°C. After washing, cells were resuspended in calcium mobilization buffer at a concentration of 1 × 10⁷ cells/ml. A flow rate of 1000 cells/sec was used to minimize washing errors. STimulation studies with the initial 24 s of each run were used to establish a baseline value for the intracellular free calcium concentration, [Ca²⁺]ᵢ. The ability of FcRγ (with or without or FcRγ/γ chain) to trigger [Ca²⁺]ᵢ increases was determined by incubating SNARF-1/Fluo-3-loaded cells with either mAb A77 or My43 for 20 min at room temperature (in the dark). After washing, cells were run on a flow cytometer for 24 s, and GAM IgG1 or GAM IgM Ab was added to cross-link FcRγ.

RNA Isolation, Reverse Transcription, and RT-PCR—Total cellular RNA was extracted from 1 × 10⁷ cells using the RNAzol B isolation method followed by cDNA synthesis (10). FcRγ chain transcripts were detected via PCR using two γ chain-specific primers, MG1 (sense 5′-CCAATCTTGGTCCCTGTC-3′) and MG2 (antisense 5′-TCACGTTCTTGCCAGACCTC-3′) (Iosgen Bioscience, Amsterdam) encompassing nucleotides 46–578 of murine γ chain. FcRγ transcripts were amplified using primers FCAL and AS1 (see above). As a control, we used a sense and antisense primer set for the hypoxanthine-guanine phosphoribosyltransferase housekeeping gene (Ref. 29; a gift from Dr. H. Savelkoul, Erasmus University, Rotterdam). Immunoprecipitations—Cells (1–2 × 10⁷ per precipitation) were washed three times in PBS before surface radioiodination by the lactoperoxidase method. Cells were lysed in 1% digitonin, 150 mM NaCl, 10 mM triethanolamine, pH 7.4, containing the protease inhibitors phenylmethylsulfonyl fluoride, N-α-tosyl-L-lysine chloromethyl ketone, soybean trypsin inhibitor, and leupeptin (15) for 45 min at 4°C. Insoluble material was removed by centrifugation at 13,000 g for 10 min.

Lysates were then precladded four times with Protein G-coated Sepharose CL-4B beads (Pharmacia) and once with beads coated with either an irrelevant mouse IgG1 mAb directed against plant allergens (CLB, Amsterdam) (A77 control) or rabbit serum (FcRγ chain antisera control). Specific precipitations were then performed with beads coated with either mAb A77 or a rabbit antisera against FcRγ chain. The beads were washed four times with digitonin lysis buffer, and precipitates were analyzed by reducing SDS-PAGE on 10% polyacrylamide gels followed by autoradiography.

Western Blotting—Proteins were immunoprecipitated as described above, separated by nonreducing SDS-PAGE on 15% polyacrylamide gels, electrophoretically transferred to nitrocellulose membranes (14). The blots were incubated in blocking buffer (PBS containing 5% nonfat dry milk) for 1 h at room temperature and then with rabbit anti-γ chain serum (1:2000) for 2 h (room temperature). After several washes, blots were incubated with horseradish peroxidase-conjugated horse antimouse IgG (1:2000; CLB, Amsterdam) in TBST (10 mM Tris, 150 mM NaCl, 0.05% Tween 20). After extensive washing, bound antibody was detected by chemiluminescence (Boehringer Mannheim, Germany).

Tyrosine Phosphorylation Assays—Transfectants were incubated with mAb A77 for 30 min at room temperature, washed twice with RPMI 1640 medium, and resuspended in separate tubes (2 × 10⁷ cells/30 μl). GAM IgG1 (final concentration 20 μg/ml) was added for the indicated time periods at 37°C to cross-link FcRγ. Reactions were stopped by the addition of 70 μl of reducing SDS-PAGE sample buffer (50 mM Tris/HCl) (pH 6.8), 10% glycerol, 4.3% SDS, 0.05% bromophenol blue, and boiled for 3 min. Blots were then washed in 60 mM Tris/HCl, pH 7.4, 120 mM NaCl, 10% glycerol, 2% SDS, and 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) before being transferred to nitrocellulose membranes.
Expression of FcαR and γ Chain in Transfected IIA1.6 B Cells—We transfected IIA1.6 B lymphoma cells with cDNAs encoding either FcαR alone, or FcαR and FcR γ chain. The FcαR was surface-expressed at a high level in both FcαR and FcαR/γ transfectants (Fig. 1A). In FcαR/γ transfectants, the presence of a γ chain message was detected by specific RT-PCR (Fig. 1B). Expression of γ chain at the protein level was confirmed by FACS analysis of permeabilized cells using an anti-γ chain serum (Fig. 1C). FcαR expressed on IIA1.6 cells were reactive with previously described CD89 mAb A77 (Fig. 1A), My43, and A59 and were capable of binding human IgA (data not shown). The increased level of FcαR expression seen in FcαR/γ cells was presumably not due to co-expression of the FcR γ chain since no increase in FcαR expression was observed following transfection of FcR γ chain cDNA to FcαR+/− IIA1.6 cells. Furthermore, these latter cells were capable of all signaling processes attributable to FcαR/γ− cells (data not shown).

We found untransfected IIA1.6 cells not to express endogenous FcR γ chain (Fig. 1B). This observation suggests that, in contrast to FcyRIIIa and FcRγ1 (12, 16), mere cell surface expression of FcαR in IIA1.6 cells is not dependent on the presence of FcR γ chain. Previously, it was also shown that FcαR could be expressed on the surface of COS cells in the absence of FcR γ chain (6). Similarly, the high affinity IgG receptor FcγRI (CD64), which also associates with FcR γ chain (13, 14), can be expressed by itself in COS (13) and 3T3 transfectants (30).

Physical Association between FcαR and γ Chain in Transfected IIA1.6 B Cells and in PMN—We next performed experiments to determine whether there is physical association between FcαR and FcR γ chain. Cells were surface-labeled with 125I and lysed in 1% digitonin prior to immunoprecipitation with anti-FcαR mAb A77 or anti-FcR γ chain antisera (Fig. 2A). Immunoprecipitation with CD89 mAb A77 resulted in the isolation of one major band of ~60 kDa (arrowheads) from both FcαR+ (lane 2) and FcαR/γ− (lane 3) transfectants, but not from untransfected IIA1.6 cells (lane 1). The molecular weight of the observed band is consistent with the predicted size of FcαR (3–5). Immunoprecipitation of radiolabeled cell lysates with an anti-γ chain-specific antibody precipitated one band of ~60 kDa only from the FcαR/γ− transfectants (Fig. 2A).

Western blotting analysis of immunoprecipitated proteins further supported the presence of a physical interaction between FcαR and FcR γ chain in transfectants and suggest that FcαR and FcR γ chain can also associate in peripheral blood PMN. Proteins were precipitated from digitonin-solubilized cells with beads coated with either A77, anti-FcR γ chain serum, or control serum (see "Materials and Methods"), transferred to nitrocellulose, and probed with anti-FcR γ chain serum. In IIA1.6 cells, a specific band of ~20 kDa, corresponding to the expected size of FcR γ chain homodimers (12), was co-precipitated by anti-FcαR mAb A77 from FcαR−/γ− cells only (Fig. 2B, lane 3). No bands of this size were precipitated by A77 from either untransfected IIA1.6 or FcαR− transfectant cell lysates (lanes 1 and 2). Similarly sized bands were detected in PMN cell lysates following immunoprecipitation with either A77 (lane 4) or anti-γ chain serum (lane 5). No specific bands of this size were seen following immunoprecipitation with either irrelevant mouse IgG1 antibody (Fig. 2C, lanes 1–4; A77 control) or with normal rabbit serum (Fig. 2C, lane 5; anti-γ chain control). The 28-kDa bands seen in Fig. 2B, lanes 1–5, are considered to be nonspecific since these bands were also seen in the control immunoprecipitations (Fig. 2C, lanes 1–5). We, furthermore, observed a slight difference in mobility of the FcR γ
Functional Association between FcR and FcR γ Chain

Fig. 2. Physical association between FcR and FcR γ chain in transfected IIA1.6 B cells and PMN. Surface radiiodinated cells were lysed in 1% digitonin lysis buffer and immunoprecipitated with either mAb A77 or anti-FcR γ chain antisera. Immunoprecipitates were separated by gel electrophoresis under reducing conditions, followed by autoradiography. A, radiolabeled cell lysates from IIA1.6 cells (lanes 1 and 4), FcR γ (lanes 2 and 5), and FcR γ/ + (lanes 3 and 6) transfecteds were immunoprecipitated with either A77 (lanes 1–3) or anti-γ chain serum (lanes 4–6). Positions of FcR γ are marked by arrowheads. B, 1% digitonin cell lysates (as indicated) were immunoprecipitated with either A77 mAb (lanes 1–4) or anti-γ chain serum (lane 5). Precipitates were separated by nonreducing SDS-PAGE, transferred to nitrocellulose, and probed with anti-γ chain serum. C, control precipitations were also performed using beads coated with an irrelevant murine IgG1 Ab (mIgG1), as isotype control for A77 (lanes 1–4), or normal rabbit serum (Rbt serum) as a control for anti-γ chain serum (lane 5). D, mouse and human FcR γ chains were precipitated from lysates of different mouse (lanes 1 and 2) or human (lanes 3 and 4) cells as detailed under “Materials and Methods.” The position of molecular mass standards are marked on the left.

chain between transfecteds (murine γ chain) and PMN (human γ chain) (Fig. 2B, lane 3 versus lanes 4 and 5). This different migration profile was surprising in view of the high homology between murine and human γ chains (12). Therefore, we next precipitated murine γ chain from FcR γ/− transfecteds and murine peritoneal macrophages, and human γ chain from PMN and U937 cells. Following SDS-PAGE and transfer to nitrocellulose, the blots were probed with anti-γ chain serum. Results presented in Fig. 2D, clearly showed a slight, albeit significant, difference in mobility between murine (lanes 1 and 2) and human (lanes 3 and 4) FcR γ chains. The observed difference in the mobilities ofhuman and mouse γ chains may be explained by the slightly different amino acid sequences of these two chains. However, the phosphorylation state of FcR γ chain has also been shown to affect its mobility in SDS-PAGE (31); therefore, differential phosphorylation between human and murine cells may also explain the observed size difference.

Previously it has been demonstrated that FcR γ chain homodimers associate with TCR-CD3, FcRRI, and all three classes of FcyRI (12–15). During the preparation of this manuscript, it was demonstrated that FcR γ chain may also be found in membrane complexes with FcRRI in the U937 cell line (17). Our results confirm this observation in a transfectant model system and, furthermore, provide evidence to suggest that FcR γ may associate with FcR γ chain in normal peripheral blood PMN.

FcR γ Chain Is Critical for FcR Signaling in IIA1.6 B Cells—We next assessed FcRRI signal transduction by both types of IIA1.6 B cell transfecteds. These FcR-negative B lymphoid cells have been used extensively as a model system for studying FcRRI functioning. IIA1.6 cells express endogenous surface IgG2a, which upon cross-linking triggers a calcium flux, protein tyrosine phosphorylation, and synthesis and release of IL-2 (9–11). Changes in [Ca2+]i and tyrosine kinase activation are important proximal signaling events and have been correlated with the presence of immunoreceptor tyrosine-based activation motifs (ITAMs) (32) within receptor cytoplasmic domains. FcRRI is devoid of ITAMs (6) while the FcR γ chain homodimer contains two such motifs (16).

Our results show FcR γ chain to be required for generation of both proximal and distal signaling events via FcRRI. Cross-linking of FcRRI in FcR γ/− transfecteds leads to a rapid rise in [Ca2+]i, that was maintained for at least 2 min (Fig. 3A). In the absence of FcR γ chain, no [Ca2+]i increase was observed. Following FcRRI cross-linking, a rapid tyrosine phosphorylation of cellular proteins was also observed only in FcR γ/− cells (Fig. 3B). Tyrosine phosphorylation was detected within 20 s and reached a maximum between 40 s and 2 min in all experiments. In all experiments, no detectable signaling responses were initiated when the cells were incubated with GAM IgG1 mAb alone, indicating that this antibody does not react with the surface IgG2a expressed by the IIA1.6 cells (as reported previously; Ref. 33).

Recently, it has been noted that FcRRI cross-linking triggers phosphorylation of FcR γ chain in U937 cells (17), supporting the hypothesis that FcR γ chain is critically involved in the generation of FcRRI signal transduction responses.

Very little is known concerning signal transduction pathways associated with FcRRI. Interestingly, it has recently been proposed that FcRRI surface expression may be regulated by [Ca2+]i, since treatment of neutrophils with known calcium agonists leads to up-regulated FcRRI expression (34). This observation, taken together with our data that FcRRI cross-linking mediates a rapid rise in [Ca2+]i, may explain the phenomena of IgA-induced FcRRI expression-up-regulation, a function apparently unique to FcRRI, at least among other FcRs (2).
FIG. 3. Signalling events triggered by cross-linking FcR α in transfected IIA1.6 B cells. A, calcium mobilization triggered by FcR α in SNARF-1/F-lys-loaded transfectants. FcR α (dotted line) or FcR α/GAM (solid line) transfectants were incubated with CD89 mAb A77 for 20 min at room temperature, and FcR α was subsequently cross-linked with GAM IgG 1 F(ab')2 (arrow). [Ca2+]i levels were analyzed by flow cytometry as described under "Materials and Methods." Data are representative of five individual experiments. B, tyrosine phosphorylation of cellular proteins upon cross-linking FcR α in FcR α−/− (left panel) or FcR α+/+ (right panel) IIA1.6 B cells. Transfectants were incubated with mAb A77 for 30 min at room temperature and washed twice with RPMI 1640 medium. Cells were then incubated with GAM IgG 1 Ab for the indicated time periods. As control, cells were also incubated either with A77 alone (A77) as negative control or with GAM IgG (GAM) to cross-link the surface IgG (positive control). Samples were separated by SDS-PAGE, transferred to nitrocellulose, and probed with an anti-phosphotyrosine mAb. C, induction of IL-2 production following cross-linking of FcR α in transfected IIA1.6 B cells. FcR α−/− (open boxes) and FcR α+/+ (filled boxes) transfectants were incubated for 24 h in wells coated with either serum IgA (IgA) or polymeric IgA (plgA). Alternatively, transfectants were incubated with CD89 mAb A77 for 30 min at room temperature, washed, and seeded into wells. A cross-linking GAM IgG 1 Ab was then added to the culture supernatant for 24 h (A77+GAM-1). Control cells were incubated with culture medium alone (medium) or had GAM γ Ab added to cross-link slgG2a (GAM). Results shown are representative of data obtained for IL-2 release in three separate experiments.

We, furthermore, examined the capacity of FcR α− and FcR α+/− transfectants to trigger the release of IL-2 from IIA1.6 B cells. Secretion of IL-2, triggered via FcR α cross-linking, also proved to be dependent on FcR γ chain co-expression (Fig. 3C). IL-2 was secreted following FcR α cross-linking by polymeric IgA, serum IgA, and CD89 mAb A77. The ability of polymeric IgA to trigger a higher level of IL-2 release than serum IgA may indicate FcR α to have higher affinity for this molecular species. IL-2 release represents a distal signaling event which can be triggered via cross-linking slgG2a in IIA1.6 B cells (35). IIA1.6 slgG2a is part of the B cell antigen receptor signaling complex which includes at least four ITAMs (36). In contrast, previous data from our laboratory utilizing a panel of FcγRII IIA1.6 transfectants showed that cross-linking of FcγRIIa (CD32; with only one ITAM) was sufficient to induce a Ca2+ response and tyrosine kinase activation but not IL-2 release (10, 32). Taken together, these data suggest the number of ITAMs within signaling complexes to be of importance for determining the type of signals generated via receptor complexes in IIA1.6 B cells. This hypothesis is supported by recent work in Jurkat cells, where the number of ITAMs within the TCR γ chain was found to quantitatively affect T cell responses (37).

Several reports in the literature suggest FcR α to be capable of synergizing with FcR γ in promoting ADCC, phagocytosis, and the respiratory burst (38–40). Our results provide a model for such co-operation in which FcR γ chain mediates signal transduction via both types of receptors. The importance of FcR γ chain in triggering phagocytosis via FcγRI has recently been demonstrated in FcγRI chain knock-out mice (41) and transfection studies (42, 43). Since our data suggest that FcR α functioning also depends on FcR γ chain association, we hypothesize that FcR γ chain-deficient mice may display aberrations in IgA-mediated mucosal immune responses.

Molecular basis for FcR α/ FdR γ chain association—Earlier reports proposed the interaction of FcR γ chain with FcγRI and FcγRIIa to involve a conserved region (LFAVDTGL) within the TM domains of these receptors, containing a negatively charged aspartic acid residue (underlined) (12). Human FcγRI (which also associates with FcR γ chain) displays high homology with FcRI and FcγRIIa within this region of the TM domain (MFLVNTVL), but lacks such an aspartic acid residue (13, 14, 44). The predicted TM domain of FcδR is 19 amino acids long and, unusually, contains a positively charged arginine residue at position 209 (Arg209) (6; Fig. 4). Moreover, FcγRI TM domain displays no obvious homology at the protein level to the TM domains of FcRI, FcγRIIa, or FcγRI (13, 14).

Although, in general, charged residues are uncommon within the TM domains of integral membrane proteins, they are not unknown. For example, the TCR-α and TCR-β chains have conserved positively charged residues within their TM domains while the invariant chains of the CD3 complex contain negatively charged TM residues. Elegant site-directed mutagenesis experiments have shown these charged residues to be of critical importance for surface expression of the TCR-CD3 complex (18–21).

Therefore, based upon the predicted TM regions of FcR α and FcγR γ chain (Fig. 4), we hypothesized the positively charged Arg209 to be important for association with FcR γ chain. To test
Functional Association between FcR and Fcγ Chain

Fig. 5. A, cells transfected with either FcR-R209D, -R209H, or -R209L and Fcγ chain were incubated with CD89 mAb My3 (curve 2) or with immunofluorescence buffer alone (curve 1) followed by FITC-conjugated GAM IgM Ab. B, detection of FcR and Fcγ chain mRNA transcripts by RT-PCR as described under "Materials and Methods." C, detection of Fcγ chain protein by Western analysis. Digitonin cell lysates (as indicated) were immunoprecipitated (IP Ab) with either anti-γ-chain serum (upper panel) or normal rabbit serum (Rt serum) as control (lower panel). Precipitates were separated by nonreducing SDS-PAGE, transferred to nitrocellulose, and probed with anti-γ-chain monoclonal antibody (Fig. 5A, B). The position of molecular mass standards are marked on the left. D, calcium mobilization triggered by mutant FcR/Fcγ chain in SNARF-1Fluor3-loaded transfectants. Transfectants were incubated with CD89 mAb My3 for 20 min at room temperature, and FcRγs were subsequently cross-linked with GAMIgM. The position of molecular mass standards are marked on the left. Data are representative of three individual experiments.

this hypothesis, we constructed mutant FcR molecules using overlap extension PCR (see "Materials and Methods"), in which the wild type Arg209 residue was replaced by either a positively charged histidine (FcR-R209H), a negatively charged aspartic acid (FcR-R209D), or an uncharged leucine (FcR-R209L). Mutant FcR cDNAs were transfected together with Fcγ chain to IIA1.6 B cells. FcR and Fcγ chain mRNA transcripts were readily detectable by RT-PCR, and the resulting mutant FcR proteins were well expressed at the cell surface as shown by FACS analysis (Fig. 5, A and B). Surprisingly, however, FcR γ chain protein was only observed in transfec-
tants co-expressing an FcR molecule possessing a positively charged residue within the TM domain, i.e. wild type FcRα and FcR-R209H (Fig. 5C). We next assayed mutant FcRαs for their ability to form functional FcRα/Fcγ chain signaling complexes, by measuring the increase in [Ca²⁺], triggered upon FcRα cross-linking (see "Materials and Methods"). Predictably, only the FcR-R209H mutant resulted in intact functional integrity of the FcRα/Fcγ chain complex comparable to the wild type FcRα (Fig. 5D). These data demonstrate that a positively charged residue within the FcRα TM domain promotes functional association with Fcγ chain. Our studies suggest furthermore that, in IIA1.6 cells, FcRα γ chain molecules unable to associate with FcRα are degraded. This occurs possibly via a mechanism recognizing the charged aspartic acid residue within the TM domain (Fig. 4). The presence of charged residues within the TM domain of some proteins can result in their retention and degradation in the endoplasmic reticulum (44).

A similar charge-based mechanism may also be operational for FcγRI-Fcγ chain association, since a positively charged histidine residue is located directly preceding the predicted TM domain (45). Charged residues located at or near the extracel-

lular/TM boundary may also be able promote association between protein subunits as suggested for the α and β subunits of the major histocompatibility complex class II molecule (46). The fact that surface expression of both FcRα and FcγRII appears independent of Fcγ chain (in contrast to FcγRI and FcγRIIIA) may, indeed, argue for a different type of Fcγ chain association between these two receptors and either FcγRI or FcγRIIIA.

In conclusion, our data demonstrate that FcRα is capable of associating with the Fcγ chain in a transfected model system and also provides evidence that these two proteins can associate in peripheral blood PMN. We, further, show FcRα signal transduction responses to be critically dependent upon co-expression of Fcγ chain, and that a positively charged residue within the TM domain of FcRα is involved in the functional association between these two molecules. Implications of these observations in terms of cooperation (and competition) between immunoglobulin receptors in cellular activation processes remain to be elucidated.

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