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Immunology

Physical association between the high-affinity IgG receptor (FcγRI) and the γ subunit of the high-affinity IgE receptor (FcεRIγ)

(Fc receptor/signaling/THP-1 cells/neutrophils)

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ABSTRACT **To investigate the structural basis of transmembrane signaling via the high-affinity IgG receptor (FcγRI), the identity of FcγRI-associated proteins in THP-1 human monocytic cells was examined. Anti-FcγRI monoclonal antibody (mAb) 197 immunoprecipitates from ^{125}I-labeled THP-1 cells solubilized in 1% digitonin buffer were found to contain a protein migrating at 12 kDa on reduction. This protein comigrated with the 12-kDa protein precipitated by the anti-high-affinity IgE receptor γ chain (FcεRIγ) mAb 4D8. Similarly, a 70-kDa band immunoprecipitated by mAb 4D8 comigrated with the 70-kDa protein band corresponding to FcγRI in mAb 197 immunoprecipitates. On two-dimensional nonreducing–reducing gel analysis, the 12-kDa protein present in both mAb 197 and mAb 4D8 immunoprecipitates migrated as a disulfide-linked homodimer. Analysis of 1% Nonidet P-40 eluates of the digitonin immunoprecipitates under reducing conditions demonstrated the presence of a 12-kDa band in the mAb 197 immunoprecipitate that could be reprecipitated with mAb 4D8. Conversely, a 70-kDa band in the mAb 4D8 immunoprecipitate could be reprecipitated with mAb 197. Similar to these findings, both mAb 197 and mAb 4D8 precipitated a 12-kDa disulfide-linked homodimeric protein from digitonin lysates of ^{125}I-labeled human neutrophils after induction of FcγRI expression with interferon γ but not from unstimulated neutrophils. Northern blot analysis confirmed the presence of FcεRIγ mRNA in interferon γ-induced human neutrophils. We conclude that FcεRIγ, a member of a family of proteins implicated in transmembrane signaling via immune recognition receptors, associates with FcγRI in human cells.**

Cross-linking of the high-affinity IgG receptor (FcγRI) by immune complexes triggers diverse functional consequences important in host defense. These functions include phagocytosis of antibody-coated targets, generation of superoxide, and transcriptional induction of cytokine genes (1). Triggering of these events by FcγRI engagement involves activation of protein tyrosine kinases, leading to phosphorylation and activation of phospholipase Cγ and generation of a Ca^{2+} flux (2, 3). The structural basis of coupling of FcγRI to protein tyrosine kinases and other intracellular biochemical signaling pathways is presently unknown.

The γ chain of the high-affinity IgE receptor (FcεRIγ) is a transmembrane protein that was first described as a subunit of the oligomeric FcεRI complex in mast cells (4, 5). FcεRIγ and the β chain of FcεRI, FcεRIβ, have since been shown to associate with the low-affinity IgG receptor (FcγRIII) in macrophages and mast cells (6–8). FcεRIγ is expressed as a disulfide-linked homodimer or as a disulfide-linked heterodimer with the closely homologous CD3ζ protein (9). In transfectant systems, coexpression of γγ, ζζ, or γζ dimers facilitates cell surface expression of FcεRI and FcγRIII (4, 10). Experiments with chimeric receptors have demonstrated that γ and ζ receptor subunits play critical roles in signal transduction and that this capacity maps to their cytoplasmic tails (11, 12).

In this report, we demonstrate the presence of a 12-kDa disulfide-linked homodimeric protein immunochemically indistinguishable from FcεRIγ in anti-FcγRI immunoprecipitates from digitonin lysates of THP-1 human monocytic cells. We also show that FcγRI and FcεRIγ are physically associated in digitonin lysates of interferon γ (IFN-γ)-treated human polymorphonuclear leukocytes. These results indicate the existence of an oligomeric FcγRI complex that contains FcεRIγ and is likely to be involved in mediating the functional effects of FcγRI ligation.

MATERIALS AND METHODS

Cell Preparations and Monoclonal Antibodies (mAbs). The monocytic cell line THP-1 was maintained in tissue culture as described (13). Sources for mAbs 197 and 32.2 (anti-FcγRI), IV.3 [anti-low-affinity IgG receptor type II (anti-FcγRII)], and 3G8 (anti-FcγRIII) and isotype controls were as described (3). The anti-FcεRIγ mAb 4D8 (IgG2b) (14) was provided by J. Kochan (Hoffmann–La Roche). Human neutrophils were isolated from fresh blood samples drawn from healthy volunteers by dextran sedimentation and Ficol–Hypaque centrifugation, as described (15). Neutrophil preparations were ≥95% pure as assessed by Giemsa staining.

Cell Surface Iodination and Immunoprecipitation. Cells ($2–5 \times 10^7$ cells per sample) were washed twice and resuspended in phosphate-buffered saline (PBS). Surface ^{125}I-labeling was then carried out using sulfosuccinimidyl-3-(4-hydroxyphenyl)propionate (Pierce) as described (16). Cells were then washed twice in PBS before lysis in Hepes/saline buffer containing 1% digitonin (Aldrich) prepared as described (17), 20 mM Hepes, 150 mM NaCl, and protease inhibitors as described (3). Lysis was carried out for 30 min on ice. Precleared lysates were subjected to immunoprecipitation with 1–2 μg of mAb per sample. Immunoprecipitates were collected on protein G-agarose (Genzyme, 20 μl per sample). In some experiments, immunoprecipitation was with antibodies or antibody fragments coupled to agarose (Affi-Gel, Bio-Rad) as described (18). Washed immunoprecipitates were analyzed by gel electrophoresis and autoradiography as described (3). For two-dimensional nonreducing/reducing gel electrophoresis, samples were electrophoresed under nonreducing conditions on 16% polyacrylamide gels (first dimension). Individual lanes were then excised from the gels, equilibrated in reducing Laemmli sample buffer for 2 h at room temperature, and applied to 16% polyacrylamide gels for electrophoresis in the second dimension.

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Abbreviations: FcγRI, high-affinity IgG receptor; FcεRI, high-affinity IgE receptor; FcγRIII, low-affinity IgG receptor type III; FcγRII, low-affinity IgG receptor type II; mAb, monoclonal antibody; NP-40, Nonidet P-40; IFN-γ, interferon γ.

Northern Blot Analysis. Isolation and analysis of total cellular RNA was performed as described (19). FcεRIγ mRNA expression was detected using a full-length FcεRIγ cDNA probe (bp 55–475) contained within an *Xho* I–*Not* I restriction fragment (a gift of J.-P. Kinet, National Institutes of Health).

RESULTS AND DISCUSSION

We (3) and others (2) have shown that engagement of FcγRI in the human monocytic cell line THP-1 results in tyrosine phosphorylation of multiple cytoplasmic proteins, including the γ-1 isoform of phospholipase C. The cytoplasmic tail of FcγRI does not contain a kinase domain, indicating that FcγRI-mediated tyrosine phosphorylation depends on coupling to a distinct tyrosine kinase. In several other receptor systems, coupling to tyrosine kinases involves additional receptor subunits containing distinct sequence motifs (20). To investigate the presence of additional FcγRI-associated subunits, surface ^{125}I-labeled THP-1 cells were lysed with digitonin under conditions known to preserve noncovalent receptor subunit associations (17). Lysates were subjected to immunoprecipitation with various mAbs and the immunoprecipitates were analyzed by gel electrophoresis and autoradiography. Fig. 1 shows the results of a representative experiment. In addition to the expected 70-kDa band, the anti-FcγRI mAb 197 precipitated a protein with an apparent molecular mass of 12 kDa under reducing conditions. This 12-kDa protein band comigrated with the 12-kDa band precipitated from THP-1 cell lysates by the anti-FcεRIγ chain mAb 4D8. In addition, mAb 4D8 precipitated a 70-kDa band that comigrated with the 70-kDa band corresponding to FcγRI in the mAb 197 immunoprecipitate. On two-dimensional nonreducing–reducing gels, as shown in Fig. 2, both anti-FcγRI immunoprecipitates and anti-FcεRIγ immunoprecipitates contained an off-diagonal spot with the characteristic migration of a disulfide-linked homodimer, with apparent molecular masses of 24 kDa in the first (nonreducing) dimension and 12 kDa in the second (reducing) dimension.

Although THP-1 cells express FcγRII and trace amounts of FcγRIII (3), involvement of these receptors in the coprecipitation of the 12-kDa protein with FcγRI could be excluded because neither IgG2a nor IgG2b myeloma proteins, which have greater affinity for FcγRII and FcγRIII than

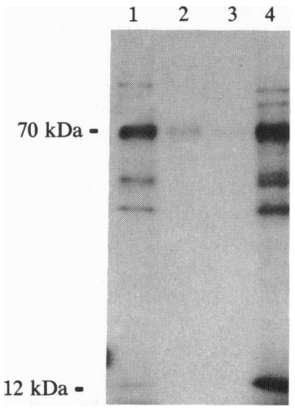

FIG. 1. A 12-kDa cell surface protein that comigrates with FcεRIγ associates with FcγRI in digitonin lysates of THP-1 cells. Cells were surface-labeled with ^{125}I and lysed in Hepes/saline buffer containing 1% digitonin. Immunoprecipitates were analyzed by gel electrophoresis and autoradiography. Lysates were immunoprecipitated with mAb 197 (lane 1), IgG2a control (lane 2), IgG2b control (lane 3), or mAb 4D8 (lane 4). Bands corresponding to FcγRI (70 kDa) and FcεRIγ (12 kDa) are indicated.

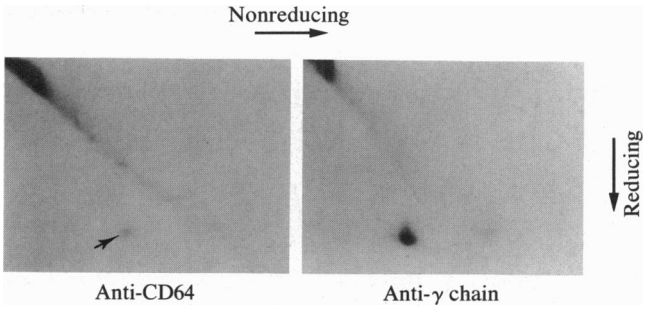

FIG. 2. Two-dimensional nonreducing–reducing gel analysis of F(ab')$_2$ mAb 32.2 and mAb 4D8 immunoprecipitates from digitonin lysates of ^{125}I-labeled THP-1 cells. Arrow indicates the position of the FcγRI-associated 12-kDa protein. Results are representative of five experiments.

murine IgG2a (the isotype of mAb 197), precipitated detectable amounts of the 12-kDa protein (Fig. 1). Similarly, no 12-kDa protein could be detected in immunoprecipitates of anti-FcγRII mAb IV.3 or anti-FcγRIII mAb 3G8 (data not shown). Finally, an affinity matrix consisting of F(ab')$_2$ fragments of the anti-FcγRI mAb 32.2 covalently coupled to agarose precipitated the same disulfide-linked homodimer as mAb 197 and mAb 4D8 (Fig. 2).

To confirm that the FcγRI-associated disulfide-linked homodimer was indeed FcεRIγ, mAb 197 immunoprecipitates were incubated in 1% Nonidet P-40 (NP-40)-containing buffer to elute weakly bound proteins, and the resulting eluates were subjected to a further immunoprecipitation step. These elution conditions were chosen because preliminary experiments indicated that there was no detectable coprecipitation of the 12-kDa protein with FcγRI by mAb 197 in 1% NP-40 lysates (data not shown). As shown in Fig. 3, incubation of the mAb 197 digitonin immunoprecipitate in 1% NP-40-containing buffer resulted in the elution of a 12-kDa protein, which could be reprecipitated by mAb 4D8 (lane 1) but not by an isotype control mAb (lane 2). Similarly, incubation of the 4D8 digitonin immunoprecipitate with 1% NP-40 resulted in the elution of a 70-kDa protein, which could be reprecipitated by mAb 197 (lane 3) but only very weakly by an isotype control mAb (lane 4). These results confirm the physical association of FcεRIγ with FcγRI in digitonin lysates of THP-1 cells.

Human neutrophils express FcγRI upon treatment with IFN-γ. We therefore examined digitonin lysates of IFN-γ-treated neutrophils for the presence of proteins coprecipitating with FcγRI. As shown in Fig. 4, two-dimensional nonreducing–reducing gel analysis of mAb 197 immunoprecipitates from lysates of IFN-γ-treated and ^{125}I-labeled neutrophils revealed the presence of a 12-kDa spot similar in migration to FcεRIγ in THP-1 cells. No similar spot was detectable in

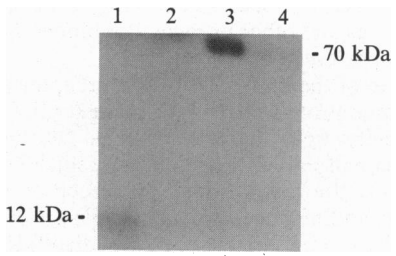

FIG. 3. FcεRIγ is the 12-kDa FcγRI-associated protein. mAb 197 and mAb 4D8 immunoprecipitates from the experiment shown in Fig. 1 were eluted with 1% NP-40, as described in text, and subjected to secondary immunoprecipitation with mAb 4D8 (lane 1) and mAb 197 (lane 3), respectively, or with isotype controls (lanes 2 and 4). Bands corresponding to FcγRI (70 kDa) and FcεRIγ (12 kDa) are indicated. Results are representative of three experiments.

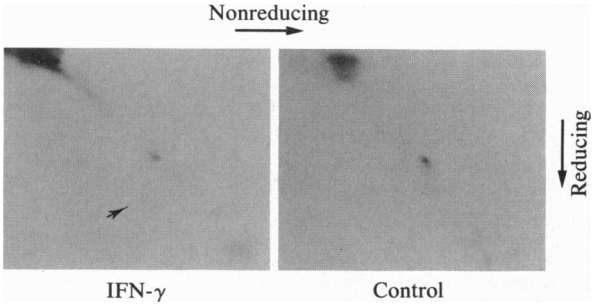

FIG. 4. A 12-kDa disulfide-linked homodimer associates with FcγRI in IFN-γ-stimulated human neutrophils. Peripheral blood neutrophils were incubated overnight in medium alone (*Right*) or medium containing IFN-γ at 400 units/ml (*Left*). Cells were then surface-labeled with ^{125}I, lysed in Hepes/saline buffer containing 1% digitonin, and subjected to immunoprecipitation with mAb 197. Arrow indicates migration of the 12-kDa off-diagonal spot.

mAb 197 immunoprecipitates of unstimulated neutrophils. Previous reports suggested that neutrophils do not express FcεRIγ (21), although to our knowledge the issue of whether FcεRIγ expression in neutrophils can be upregulated by IFN-γ has not been explicitly addressed. Fig. 5*A* shows that the anti-FcεRIγ mAb 4D8 did indeed precipitate a protein with the expected migration for FcεRIγ from IFN-γ-treated neutrophils. As shown in Fig. 5*B*, Northern blot analysis of total RNA prepared from untreated and from IFN-γ-treated neutrophils demonstrated that IFN-γ strongly induced the appearance of an RNA transcript that hybridized with a ^{32}P-labeled FcεRIγ cDNA probe. The size of this transcript (≈0.75 kb) was the same as that present in peripheral blood mononuclear cell RNA and the same as that reported (22) for human FcεRIγ transcripts. The most likely explanation of these results is that IFN-γ induces the expression of both

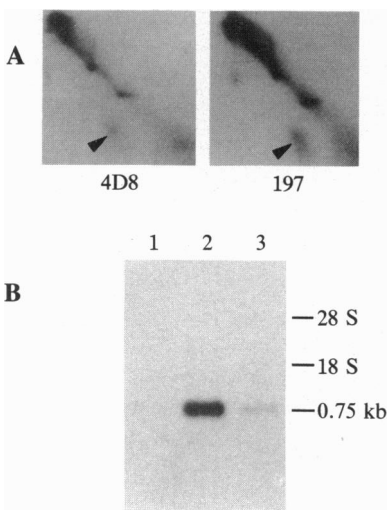

FIG. 5. FcεRIγ expression in IFN-γ-treated neutrophils. (*A*) Peripheral blood neutrophils incubated overnight in medium containing IFN-γ at 400 units/ml were subjected to immunoprecipitation with mAb 4D8 (*Left*) or with mAb 197 (*Right*) and analyzed on nonreducing–reducing gel, as in Figs. 2 and 4. The 12-kDa spot (arrowhead) corresponding to FcεRIγ was not precipitated by a control IgG2b mAb (data not shown). (*B*) Northern blot analysis of hybridization of ^{32}P-labeled FcεRIγ probe to total RNA extracted from neutrophils cultured overnight in medium alone (lane 1) or in medium containing IFN-γ at 400 units/ml (lane 2) or from peripheral blood mononuclear cells (lane 3). The blot was washed under stringent conditions prior to autoradiography. Lanes were loaded with equivalent amounts of RNA as judged by hybridization of a β-actin probe (data not shown). Results are representative of three experiments.

FcεRIγ and FcγRI and that FcεRIγ associates with FcγRI in human neutrophils. Less probably, the 12-kDa FcγRI-associated protein in neutrophils could represent a distinct protein having a high degree of homology with FcεRIγ at both protein and DNA levels.

The association between FcγRI and FcεRIγ may be mediated by interactions between transmembrane residues. It was previously reported that mutants of FcγRIIIA and CD3ζ that lack cytoplasmic tails can still associate with one another, whereas mutations of single transmembrane amino acid residues can markedly reduce their association (10). As shown in Fig. 6, the predicted transmembrane sequence of FcγRI shares strong homology with the transmembrane sequences of both FcγRIIIA and FcεRI. This homology suggests that FcγRI, like FcγRIIIA and FcεRI, may associate with FcεRIγ via transmembrane domain protein–protein interactions.

In addition to FcεRIγ, both the FcεRI and the FcγRIII complexes in mast cells contain a type II membrane protein, FcεRIβ (8, 23). In the experiment shown in Fig. 1, there is no evidence of an FcγRI-associated protein in the expected molecular mass range of FcεRIβ (25–30 kDa). Furthermore, monocytic cell lines have been reported to be negative for FcεRIβ by Northern blot analysis (23). It is still possible that a type II membrane protein homologous to FcεRIβ forms part of an FcγRI complex, since the intracellular N terminus of such a protein would be inaccessible to labeling with the iodination reagent used in the present study, which labels proteins on extracellular free amino groups (16).

In a previous study on FcγRIII subunit associations, it was noted that anti-FcγRIII immunoprecipitates from mast cells contain disproportionately small amounts of FcεRIγ compared to anti-FcεRIγ immunoprecipitates (8). Similar to this, we found a much less intense FcεRIγ band in anti-FcγRI immunoprecipitates than in anti-FcεRIγ immunoprecipitates. This may reflect the stoichiometry of the association of FcεRIγ with FcγRI. Alternatively, the anti-FcγRI mAb used for immunoprecipitation may partially inhibit binding of FcεRIγ to FcγRI, either by steric hindrance or by an induced conformational change.

As demonstrated in Figs. 1 and 2, mAb 4D8 strongly precipitated a 70-kDa FcγRI band from digitonin lysates of THP-1 cells. This could reflect the fact that mAb 4D8 was developed for its ability to preferentially precipitate intact IgE-ligated FcεRIγ complexes (14). The isotype of mAb 4D8 (IgG2b) has relatively weak affinity for FcγRI, as confirmed by the finding that murine IgG2b myeloma protein precipitated a 70-kDa protein from THP-1 cell lysates only weakly (Fig. 1). Nevertheless, we could not rule out the possibility that the Fc portion of 4D8 contributed to its ability to precipitate FcγRI. We attempted to generate F(ab')$_2$ fragments of mAb 4D8, but this mAb appears to be unusually

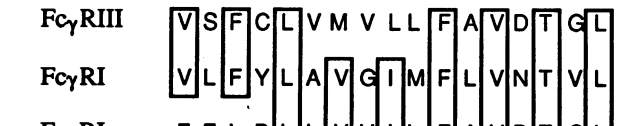

FIG. 6. Sequence homology within the transmembrane domains of FcγRI, FcγRIIIA, and FcεRI. Comparison of a 17-amino acid sequence within the predicted transmembrane domain of FcγRI with corresponding sequences from the predicted transmembrane domains of two Fc receptors, FcγRIII and FcεRI, that associate with the CD3ζ and FcεRIγ chains. Sequences were accessed from GenBank (December 1992) and aligned using the BESTFIT algorithm (GCG). The amino acid sequences for residues 209–225 of FcγRIII, residues 293–309 of FcγRI, and residues 180–196 of FcεRIα are shown. Boxed residues are identical in FcγRI and one or both of the other two Fc receptors as shown.

sensitive to papain digestion and only inactive fragments were obtained (data not shown).

Experiments using receptor chimeras have mapped signal transduction functions of FcεRIγ and CD3ζ, including coupling to tyrosine kinases, to sequence motifs in their cytoplasmic tails first identified by M. Reth (24). Reth motifs have the consensus sequence $YX_2LX_7YX_2(L/I)$ and are found in the cytoplasmic tails of several other hematopoietic cell receptor subunits (including CD3γ, δ, and ε, Ig-α, and Ig-β) and FcεRIβ. A closely related motif is also found in the cytoplasmic tail of FcγRII (25). Thus, these observations and the coassociation of FcεRIγ with FcγRI strongly suggest that FcεRIγ mediates signaling via FcγRI. This would imply a striking similarity between identified signaling mechanisms utilized by FcγRI, FcγRII, and FcγRIII, despite differences in the functional consequences of their ligation (26). The signaling mechanisms that determine the distinct effector functions of FcγRI remain to be determined.

Note Added in Proof. Physical association between FcεRIγ and FcγRI in U937 cells was recently reported by Ernst *et al.* (27).

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