Direct interaction between FcγRI (CD64) and periplakin controls receptor endocytosis and ligand binding capacity

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FcγRI depends for its biological function on both the intracellular domain of the α-chain and associated Fc receptor (FcR) γ-chains. However, functional protein effectors of FcγRI’s intracellular domain have not been identified. In this study, we identified periplakin (PPL) as a selective interacting protein for the intracellular tail of FcγRI but no other activatory FcRs. The interaction was confirmed by coimmunoprecipitation and blot-overlay assays. PPL and FcγRI colocalized at the plasma membrane in monocytes and cell transfectants, and both were up-regulated by IFN-γ. By expressing C-terminal PPL in transfectants, we established a pivotal role for this protein in FcγRI ligand binding, endocytosis, and antigen presentation. These data illustrate that intracellular protein interactions with a multisubunit FcR α-chain can confer unique properties to the receptor.

Antibody (Ig) complexes can induce potent immune effector functions by crosslinking Fc receptors (FcRs) (reviewed in refs. 1 and 2). Multiple FcRs exist as complexes of unique Ig-binding α-chains that associate with prominent signaling subunits harboring immunoreceptor tyrosine-based activation motifs. The FcR γ-chain associates with a variety of FcR α-chains that lack known signaling motifs, and it is crucial for induction of phagocytosis and NADPH oxidase assembly, for example (3–5). These observations have led to the paradigm that multisubunit FcRs do not signal through the cytosolic domains (CYS) of their α-chains, but signal through the FcR γ-chain. However, there is considerable evidence that CYS’s of particular FcR α-chains modulate FcR receptor function through as-yet-undocumented signaling pathways.

FcγRI is a high-affinity IgG receptor with a strict myeloid cell distribution that is up-regulated during inflammation (6, 7). Its in vivo role is illustrated by FcγRI−/− mice that exhibit impaired Ab-dependent cellular processes such as bacterial clearance, phagocytosis, antigen presentation, and cytokine production (8, 9).

Within the multisubunit FcR family, it has been shown that the FcγRI α-chain induces effector functions. The FcγRI cytosolic domain (FcγRI-CY) mediated MHC class II antigen presentation without active FcR γ-chain signaling (10), whereas deletion of FcγRI-CY retarded kinetics of endocytosis and phagocytosis and abrogated FcγRI-triggered IL-6 secretion (11). Thus far, only actin-binding protein 280 (filamin A) has been described to bind FcγRI-CY under some conditions, but effects on receptor function have not been shown (12).

Here, we identified periplakin (PPL) to selectively interact with FcγRI-CY in yeast two-hybrid screens. PPL is a cytosolic protein of 195 kDa and a member of the cytoskeletal-associated plakin family (reviewed in ref. 13). Plakins contribute to the structural integrity of epithelia by, e.g., tethering cytoplasmic domains of adhesive receptors to the cytoskeleton. PPL is associated with desmosomes in keratinocytes and is involved in cornified envelope assembly (14–16). PPL’s central rod domain mediates homo- and heterodimerization and separates the N terminus (which can associate with actin filaments) from the vimentin-interacting C terminus (17–19). Additionally, recent studies suggested that PPL is involved in signaling through protein kinase B and G proteins in nonimmune cells (20, 21). We found PPL expression in myeloid cells, and expression was increased by IFN-γ. Immunoprecipitation of transfected IIa/1.6 cells and binding assays with recombinant proteins confirmed the interaction between FcγRI and PPL. Furthermore, we assessed the role of PPL in FcγRI-mediated ligand binding, receptor modulation, and antigen presentation.

Materials and Methods

**Generated Constructs.** cDNAs encoding the cytosolic tail sequences of human FcγRI, FcγRIIa, FcγRIIia, FcγRI, FcγRI, and murine (m) FcγRI were inserted in pGBT9. Casein kinase II β-subunit was cloned into pGAD-GH. Full-length (fl) FcγRI was cloned into pcDNA3, the cytosolic tail of FcγRI also in pGEX-2T (Amersham Biosciences). The mFcR γ-chain and mutant γY65F,γ76F were inserted in a pNUT vector described in ref. 10. cDNAs encoding PPL clone 3.4 were inserted in pGBT9, pcDNA3.1/His (Invitrogen, Leek, The Netherlands), and pGEX-2T, from which the GST tag had been replaced by a His tag (six histidine residues). fl-PPL was in pBluescript (a kind gift of F. M. Watt, Keratinocyte Laboratory, London Research Institute, London). cDNAs generated by PCR were verified by sequence analysis.

**Yeast Two-Hybrid Screening.** Oligo(dT)-primed HeLa (pGAD-GH; gift of the Cell Biology Department of University Medical Center Utrecht) and bone marrow (pACT-2) cDNA libraries were screened for FcγRI-CY-interacting proteins in yeast strain YGH1 (all from Clontech). Protein interactions were assessed by growth of colonies on histidine-depleted medium, and β-galactosidase activity was assessed with a replica filter assay. FcγRI-interacting proteins and FcγRI signaling motifs were identified by scanning cDNA sequences with BLAST (www.ncbi.nlm.nih.gov/BLAST).

**RT-PCR.** FcγRI was amplified from oligo(dT)-primed cDNA by 25 PCR cycles (30 s at 95°C, 30 s at 57°C, and 45 s at 72°C) by using primers annealing to FcγRI-CY (5’-CTGTTTCTCTGGGTGAGACAAATACG-3’ and 5’-AGAAGTGTGTCTCATGAGTAT-3’). PPL PCRs were performed for 35 cycles (30 s at 95°C, 30 s
at 52°C, and 1 min at 72°C) with primers annealing to human and mouse PPL (5′-AGCCAAGAGATCCAG-3′ and 5′-CTACTTGTGCCCAG-3′). The presence of cDNA in each sample was verified by GAPDH PCR.

**Intracellular PPL Staining.** Human peripheral blood monocytes were isolated from buffy coats of healthy volunteers. Mononuclear cells were collected from Ficoll gradients and cultured with Iscove’s modified Dulbecco’s medium containing l-glutamine ( Gibco/BRL) supplemented with 10% FCS, penicillin, and streptomycin. After 3 h at 37°C, nonadherent cells were removed and adherent cells were incubated overnight with 300 IU/ml IFN-γ (IFN-γ, Boehringer Ingelheim, Biberach, Germany). Monocytes and II.A1.6 cells were fixed in PBS with 3.3% paraformaldehyde and stained in PBS containing 0.2% BSA, 0.1% saponin, 5% mouse serum, and 5% goat serum. PPL was detected by rabbit serum 5117 (characterized in ref. 20 and kindly provided by B. Burgering, University Medical Center Utrecht) diluted 100-fold and subsequently by goat-anti-rabbit-FITC (Jackson Immunoresearch). Preimmune serum was used as negative control.

**Confocal Immunofluorescence Microscopy.** Monocytes were isolated as described above, resuspended in PBS with 2 mM EDTA and 0.1% BSA, washed in medium, adhered to poly(L-lysine) slides, and processed for immunofluorescence as described in ref. 22. Aspecific interactions were blocked by the addition of 5% mouse serum and 5% goat serum during staining. FcγRI was stained with mAb m22-FITC (Medarex, Annandale, NY) or 10.1-FITC (Serotec), and PPL was stained with rabbit serum 5117 (characterized in ref. 20 and kindly provided by B. Burgering, University Medical Center Utrecht) diluted 100-fold and subsequently by goat-anti-rabbit-FITC (Jackson Immunoresearch). Preimmune serum was used as negative control.

**Immunoprecipitation.** II.A1.6 cells were stably transfected with FcγRI and FcγRII as described in ref. 24, and C-terminal PPL was transiently transfected by electroporation. After 48 h, homogenates of 10⁶ cells per immunoprecipitation in 500 μl of radioimmuno precipitation assay (RIPA) buffer (150 mM NaCl/100 mM Tris-HCl, pH 8.3/0.1% SDS/0.5% deoxycholate/1% Triton X-100) were spun for 10 min at 1,500 × g, and supernatants were incubated with protein A beads preabsorbed with 7445 serum (PPL-specific rabbit serum, B. Burgering; ref. 20). After a 1-h rotation at 4°C, beads were washed four times with RIPA buffer and boiled in reducing Laemmli sample buffer. Samples were analyzed by Western blotting by using rabbit serum to detect FcγRII. Samples were analyzed by Western blotting by using rabbit serum to detect FcγRII (no. 3532, kindly provided by R. Kimberly, University of Alabama at Birmingham, Birmingham) and rabbit serum 5117 to detect PPL.

**Coprecipitation of GST-FcγRI and His-PPL.** Fusion protein of FcγRI-CY and GST (GST-FcγRI) or His-tagged PPL clone 3.4 (His-PPL) were purified from nonnaturated Escherichia coli lysates with gluthathione Sepharose 4B (Amersham Biosciences) and Ni-NTA Spin columns (Qiagen, Hilden, Germany), respectively. Tosyl-activated M-280 Dynabeads (Dynal, Oslo) were precoated with anti-GST Ab (Amersham Biosciences) according to the manufacturers’ instructions. Beads were incubated overnight with 1 μg of GST-FcγRI or GST and 1 μg of His-PPL in 500 μl of RIPA buffer. Beads were washed three times in RIPA, boiled in Laemmli sample buffer, and analyzed by SDS/PAGE and Western blotting.

![Fig. 1. PPL binds the FcγRI cytosolic tail in yeast two-hybrid screens.](image-url) (A) Protein sequences of the cytosolic tails of FcγRI, FcγRIIa, FcγRIIb, FcγRI, FcγRII, and mFcγRI are depicted; putative PKC (dotted line) and casein kinase II (solid line) recognition motifs are indicated for FcγRI. Amino acids are color-coded as follows: green, hydrophobic/ aromatic; blue, basic/hydrophilic; red, acidic; yellow, aliphatic. Predicted regions (PredictProtein at www.embl-heidelberg.de) are indicated. TM, transmembrane. Numbers at the top refer to amino acids of FcγRI, and numbers at the bottom indicate the size of the predicted cytoplasmic domain. (B–D) Yeast two-hybrid analyses. For each combination of cDNA, three independent colonies were transferred to histidine-lacking plates and tested for β-galactosidase activity (indicated by blue staining). (B) Protein interactions of FcγRI-CY with PPL. CDNA clones 2.2, 1.77, 3.4, 4.27, and 91 and casein kinase II. (C) Protein interactions of PPL clone 3.4 with FcγRI-CY that lacks residues 311–313 (vTδ), FcγRI with a spacer of six glycines between Galα4-8D and FcγRII-CY (6×Gly/FcγRII), and PPL 3.4. (D) Protein interactions of PPL clone 3.4 with a panel of activatory FCRs. One representative experiment of three is shown. (E) A hypothetical drawing of a PPL dimer. The five independent hits from yeast two-hybrid screens are indicated below the drawing.
Blot Overlay for GST-FcRI and His-PPL. Purified fractions of His-tagged PPL clone 3.4 were separated by SDS/PAGE and transferred to poly(vinylidene difluoride) membranes. Membranes were blocked with 5% low-fat milk powder in PBS and incubated overnight with 50 nM purified GST-FcyRII or GST in 3 ml of RIPA buffer supplemented with 0.1% BSA and 0.5% low-fat milk powder. Membranes were washed in RIPA buffer, and binding of GST-Fc-yRI was detected by incubation with anti-GST Ab (Amersham Biosciences) and rabbit anti-goat IgG Ab conjugated to horseradish peroxidase (Pierce), followed by enhanced chemiluminescence and autoradiography. Methodology was adapted from refs. 25 and 26.

Modulation of FcRI Expression. Rabbit IgG–ovalbumin (OVA) complexes were generated as described (10). A total of 2.5 × 10^6 transfected IIA1.6 cells were transferred to 96-well plates in 100 μl of RPMI medium 1640 with 10% FCS and increasing concentrations of rabbit IgG–OVA complexes. Samples were incubated at 37°C for 16 h, washed, and stained with CD64 mAb 32.2 (Medarex; ref. 27), followed by phycoerythrin-labeled F(ab’)2 of goat anti-mouse IgG1 antiserum (Jackson ImmunoResearch). Modulation of Fc-yRI was determined by flow cytometry as the reduction in receptor expression relative to untreated transfectants (28).

Antigen Presentation Assays. Antigen presentation assays were carried out as described in ref. 29. Briefly, IIA1.6 transfectants were incubated with different concentrations of immune complexes and cocultured with OVA-specific DO11.1 T cells that produce IL-2 after T cell receptor triggering. IL-2 in supernatants is indicated by the proliferative response of IL-2-dependent CTLL-2 cells measured by [3H]thymidine incorporation. To assess receptor-independent antigen presentation, a large excess of OVA (100 μg/ml) was added to each IIA1.6 cell line, which was set at 100%. [3H]Thymidine incorporation was expressed as percentage of this maximum level.

Erythrocyte–Antibody (EA) Rosette Assay. Human erythrocytes were prepared by Ficol/Hypaque density centrifugation and stored in sterile Alsever’s solution at 4°C. Erythrocytes were opsonized by serial dilutions of hybridoma supernatants containing mIgG2a anti-human glycophorin A mAb (30) for 1 h at 2 × 10^6 erythrocytes per ml at 4°C. Erythrocytes were washed, and 5 × 10^6 erythrocytes were resuspended with 1 × 10^6 transfected IIA1.6 cells in 50 μl of RPMI medium 1640 in round-bottom 96-well plates and incubated for 60 min at 4°C. EA rosettes were resuspended after 30 and 60 min and fixed by addition of 3% paraformaldehyde for 30 min. Cells were diluted 2- to 3-fold in Heps-buffered RPMI medium 1640, and bound erythrocytes were counted by light microscopy. Expression levels of Fc-yRI were measured by flow cytometry using F(ab’)2 of anti-Fc-yRI mAb H22 and goat F(ab’)2 anti-human κ light chain-FITC (Southern Biotechnology Associates).

Results and Discussion

Fc-yRI Cytosolic Tail Interacts with PPL. Alignment of intracellular domains of FcR did not show high sequence similarity (Fig. 1A). Fc-yRI-CY was found relatively large for non-tyrosine-containing CYs and shared highest sequence similarity with Fc-yRIIA (only 20%, compared with 19% for mFc-yRI and 12% for Fc-yRIIIA). No functional signal sequences were recognized within Fc-yRI-CY, but two frequently observed phosphorylation sites of casein kinase II and one of PKC were predicted in Fc-yRI-CY. However, interactions of Fc-yRI-CY with the β-subunit of casein kinase II were not observed (Fig. 1B).

We identified PPL as a selective Fc-yRI-CY-interacting pro-

Fig. 2. Expression of PPL in leukocytes. (A) RT-PCRs were performed for Fc-RI, PPL, and GAPDH with RNA of MCF7, IIA1.6, Jurkat, Raji, and U937 cell lines, primary human polymorphonuclear cells, and Fc-RI (transgenic Tg) and nontransgenic (NTg) littermates] murine bone marrow-derived dendritic cells (DC) (39). IFN-γ (300 units/ml) stimulations were overnight. (B) Flow cytometric analysis of PPL expression in primary monocytes. Dotted trace, preimmune serum background staining; open solid trace, PPL levels in freshly isolated monocytes; shaded trace, PPL levels after overnight IFN-γ stimulation. (C) Confocal microscope analyses of monocytes, cultured overnight with or without IFN-γ, adhered to poly(l-lysine) coated slides, and fluorescently labeled for Fc-RI (mAb m22-FITC) and PPL (rabbit serum 5117/goat anti-rabbit-CY3). Merged pictures show colocalization in yellow. Alternatively, IFN-γ-stimulated monocytes were incubated with the FITC-labeled anti-Fc-RI mAb H22 for 1 h and stained for PPL. (D) Subcellular distribution of Fc-RII and PPL in IIA1.6 cells. PPL (fl or clone 3.4) was transiently expressed in IIA1.6 cells stably transfected with WT Fc-RII or mutant Fc-RII-A332 (which lacked residues 333–374) and WT Fc-RI (γ-chain [γ]) or γY65F, Y76F. Fc-RI was stained with 10.1-FITC. PPL is shown in red, and merged pictures of two representative cells show colocalization in yellow (n = 3).
tein in four independent yeast two-hybrid screens of HeLa (clones 2.2 and 3.4) and bone marrow cDNA libraries (clones 1.77, 4.27, and 4.91) (Fig. 1 B-D). Notably, deletion of the first three amino acids of our FcγRI-CY construct (-VTI) abrogated the interaction (Fig. 1C), implicating these to be part of the PPL binding site of FcγRI. This finding would suggest a larger cytosolic region than is indicated by computation. Interactions were not disturbed when a glycine kinked was cloned between Gal4-binding domain (Gal4-BD) and FcγRI-CY but were abolished when Gal4-BD was expressed without the FcγRI insert (Fig. 1D, empty). No binding to any other FcγR was detected, including mFcγRI (Fig. 1D), which is 32% identical and 56% similar in the proximal part of FcγRI-CY. Furthermore, we confirmed the capacity of PPL to form homodimers as has been suggested (14). The topology of PPL and location of the five clones identified by yeast two-hybrid screens are indicated in Fig. 1E.

**PPL Is Expressed in Myeloid Cells.** The cellular distribution of PPL was determined by RT-PCR and was found highly similar to FcγRI (Fig. 2A). U937 cells, activated human granulocytes (polymorphonuclear cells), and bone marrow-derived dendritic cells from FcγRI transgenic mice (31) expressed transcripts, but these were hardly, if at all, detected in lymphoid cell lines (Jurkat and Raji). The myeloid/lymphoid IIA1.6 cells (24) expressed some endogenous PPL (Fig. 2A; see also Fig. 6, which is published as supporting information on the PNAS web site). Increased levels of FcγRI and PPL were found upon overnight IFN-γ treatment (Fig. 2A and B). An Sp1 site is present in the promoter region of PPL and could regulate its myeloid expression, because Sp1 is involved in expression of myeloid markers such as CD11c (32, 33).

**Plasma Membrane Localization of FcγRI and PPL.** Next, we studied the subcellular localization of both proteins in primary monocytes and transfectants. Cells were stained for FcγRI (green) and PPL (red) and examined by using confocal scanning laser microscopy. Both proteins colocalized (indicated by the yellow color) at the plasma membrane in specific patches in monocytes (Fig. 2B). IFN-γ incubation enhanced detection of colocalization and increased PPL staining intensity, as was indicated by intracellular stainings of PPL by flow cytometry (Fig. 2B). Intracellular staining of FcγRI and to some extent PPL was also observed but did not specifically colocalize. When FcγRI was triggering with the FITC-labeled anti-CD64 mAb H22, distinct staining patterns were observed for internalized FcγRI and PPL that relocated to the cells’ interior (Fig. 2C). These data suggested that PPL acts before or at early moments of FcγRI triggering. IFN-γ treated polymorphonuclear cells were also stained, but high background fluorescence prevented specific detection of PPL (data not shown).

In IIA1.6 transfectants (Fig. 2D), fl-PPL and C-terminal PPL (c-PPL) colocalized with FcγRI at the plasma membrane as in monocytes. Active or mutated (γY65F,γY76F) FcγRI chain did not affect staining patterns. However, deletion of FcγRI residues 333–374 (FcγRIΔ332) induced clear green and red patches at the plasma membrane, suggesting that this mutant receptor does not interact with PPL.

**FcγRI and PPL Coimmunoprecipitate in Immune Cells and Interact Directly.** The B cell/macrophage cell line IIA1.6 (24) is devoid of endogenous FcRs (34) and has been used previously in functional assays for FcγRI (10). We performed transient transfections with the C-terminal part of PPL in IIA1.6 cells stably expressing FcγRI, with or without Fcγ-γ-chains (Fig. 3A). Immunoprecipitations were performed with Abs to PPL, and

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**Fig. 3.** Physical interaction between FcγRI and PPL. (A) PPL clone 2.2 was transiently expressed in IIA1.6 cells stably transfected with FcγRI with (Left) or without (Right) the Fcγ-γ-chain. Cells were lysed in RIPA buffer after 48 h, and homogenates were incubated with a rabbit anti-PPL antiserum (5117) or control serum. Pre- and post-PPL immunoprecipitated supernatants, anti-PPL, and control beads were subjected to SDS-PAGE, blotted onto polyvinylidene fluoride membranes, and stained for PPL (upper) and FcγRI (Lower). One representative experiment of three is shown. (B and C) Recombinant FcγRI-CY (GST-FcγRI) and PPL clone 3.4 (His-PPL) were purified and assessed for interaction by immunoprecipitation (B) or blot overlay (C). (B) His-PPL was precipitated with anti-GST beads in the presence of GST-FcγRI but not GST tag alone. Membranes were incubated with anti-His Ab and subsequently with anti-GST Ab. (C) Blot-overlay analysis. Lanes 1 and 2, Coomassie stainings of purified His-PPL fractions containing ~1 and 0.3 µg of His-PPL. Lanes 3 and 4, His-PPL was transferred to membranes and stained by anti-His Ab. Lanes 5–8, Blots were overlaid with GST-FcγRI (lanes 5 and 6) or GST tag alone (lanes 7 and 8), and binding was detected by subsequent incubations with anti-GST Ab. Lanes 7 and 8 were overexposed compared with lanes 5 and 6. One representative experiment of three is shown.
Western blots of these were stained for PPL and FcRI independent of the FcγRI, as the FcγRI, FcγRIIγ-chain (WT or FcγRIIγ-chain Y65F,Y76F, and C-terminal PPL clone 3.4) were incubated with membranes and incubated with OVA-specific D011 T cells for 16 h. IL-2 production of D011 cells was measured by [3H]thymidine incorporation after treatment with 100 μg of OVA was set at 100% for each transfectant. Two independent stable transfectants for FcγRII and γ65F,76F (designated 1 and 2) were compared with two stable transfectants for FcγRI, γ65F,76F, and C-terminal PPL clone 3.4. Untransfected IIA1.6 cells were used as control. Measurements were in triplicate, error bars mark standard deviations, and one representative experiment of three is shown. Groups were compared by Student’s paired t test, which indicated statistical difference (P < 0.05).

C-Terminal PPL Enhances Downstream Effector Functions of FcγRI-CY. Upon C-terminal PPL transfection, no appreciable differences in cell-surface expression were observed in flow cytometric and microscopic analyses (data not shown). Next, we tested the capacity of C-terminal PPL to affect FcγRI function using subcloned IIA1.6 transfectants. Receptor modulation is defined by plasma membrane reduction of FcγRI after 16 h of incubation with immune complexes and is indicative of lysosomal trafficking of FcγRI/antigen complexes (28). Typically, 20–30% of the receptor disappeared after overnight incubation with 1 μg/ml immune complexes, independent of active or signaling “dead” (γ65F,76F) FcγRIIγ-chains (Fig. 4A). Stable expression of C-terminal PPL in FcγRI transfectants (confirmed by Western blot; data not shown) increased levels of internalized receptors to 50–60% (P < 0.05, paired Student’s t test; n = 4).

In this transfection model, FcγRI-CY mediates antigen presentation in the presence of γ65F,76F, although antigen presentation is suboptimal (~60% left) when compared with the WT FcγRI γ-chain (10). We confirmed the capacity of FcγRI to mediate antigen presentation by comparing FcγRI-dependent antigen presentation to MHC class II presentation after pinocytosis of a large excess of free OVA (set at 100%; Fig. 4B). Stable coexpression of C-terminal PPL in these transfectants increased antigen presentation (P < 0.05, paired Student’s t test; n = 3), as was shown for FcγRI modulation. Untransfected cells exhibited no response to these concentrations of OVA-immune complexes, implicating FcγRI dependency. Together, these results suggested that PPL regulates downstream effector functions of FcγRI-CY. 

C-Terminal PPL Enhances FcγRI Ligand Binding. Because PPL seemed to interact with FcγRI without receptor crosslinking (Figs. 2C and 3A), we tested the ability of C-terminal PPL to affect FcγRI ligand binding. Ligand binding was increased by C-terminal PPL, as illustrated by enhanced EA rosette scores (Fig. 5), although FcγRI expression levels were similar. These experiments were performed at 4°C to inhibit cellular activity and thus indicated that PPL controls FcγRI function by mechanisms that were preexperimentally active in nonstimulated cells. To credit...
temperature sensitivity of protein interactions, similar experiments were performed at a physiological temperature (37°C) yielding identical results, also in the presence of okadaic acid, which prevents FcγRI-mediated phagocytosis (35) (see Fig. 7, which is published as supporting information on the PNAS web site).

The effect of C-terminal PPL is likely by functional blockade of FcγRI–endogenous PPL interaction in IIA1.6 cells. Although we could not detect endogenous PPL by Western blot, flow cytometric analysis indicated PPL protein in IIA1.6 (Fig. 6). Similarly, transduction of cells with PPL peptides containing a minimal FcγRI binding domain enhanced FcγRI ligand binding comparable to the effect of C-terminal PPL (36). Thus, endogenous mPPL (clone 3.4 shared 94% similarity with its murine counterpart) bound FcγRI, and this was blocked by C-terminal PPL. This finding would imply that endogenous PPL lowers ligand binding. Miller et al. (37) found that FcγRI-CY removal increases ligand affinity, concordant with our hypothesis.

In vivo, regulation of PPL function might lower ligand affinity to facilitate replacement of bound monomeric IgG for antigen-complexed IgG. FcγRI m− mice illustrated that serum IgG functionally competes with ligand complexes for induction of cellular activation, indicating that lower affinity might be beneficial for immune complex triggering (8). Because cells with intact FcγRI–PPL interaction exhibit lower capacity to form EA rosettes, PPL expression might also skew interactions of immune cells to more intensely opsonized antigens.

Concluding Remarks. Signal transduction by multichain FcRs has been considered to be exclusively mediated by immunoreceptor tyrosine-based activation motif-containing subunits. However, we found PPL to selectively modulate FcγRI function through its inside-out signaling. Enhanced ligand binding through inside-out signaling has been described for FcεRI (38). Here, we document a similar mechanism for FcγRI and the protein that is involved in this inside-out mechanism.

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