

A Common Site of the Fc Receptor γ Subunit Interacts with the Unrelated Immunoreceptors Fc α RI and Fc ϵ RI*

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The transmembrane (TM) region of the Fc receptor- γ (FcR γ) chain is responsible for the association of this ubiquitous signal transduction subunit with many immunoreceptor ligand binding chains, making FcR γ key to a number of leukocyte activities in immunity and disease. Some receptors contain a TM arginine residue that interacts with Asp-11 of the FcR γ subunit, but otherwise the molecular basis for the FcR γ subunit interactions is largely unknown. This study reports residues in the TM region of the FcR γ subunit are important for association with the high affinity IgE receptor Fc ϵ RI and a leukocyte receptor cluster member, the IgA receptor Fc α RI. FcR γ residue Leu-21 was essential for surface expression of Fc ϵ RI α/γ_2 and Tyr-8, Leu-14, and Phe-15 contributed to expression. Likewise, detergent-stable FcR γ association with Fc α RI was also dependent on Leu-14 and Leu-21 and in addition required residues Tyr-17, Tyr-25, and Cys-26. Modeling the TM regions of the FcR γ dimer indicated these residues interacting with both Fc α RI and Fc ϵ RI are near the interface between the two FcR γ TM helices. Furthermore, the FcR γ residues interacting with Fc α RI form a leucine zipper-like interface with mutagenesis confirming a complementary interface comprising Fc α RI residues Leu-217, Leu-220, and Leu-224. The dependence of these two nonhomologous receptor interactions on FcR γ Leu-14 and Leu-21 suggests that all the associated Fc receptors and the activating leukocyte receptor cluster members interact with this one site. Taken together these data provide a molecular basis for understanding how disparate receptor families assemble with the FcR γ subunit.

The Fc receptor γ subunit (FcR γ)² is a ubiquitous signal transduction subunit widely found in hematopoietic cells and is present on macrophages, monocytes, dendritic cells, natural killer cells, platelets, eosinophils, mast cells, $\gamma\delta$ T cells, and CD4 $\alpha\beta$ effector T cells (1, 2). It is a promiscuous receptor subunit originally identified as a subunit of the high affinity IgE receptor Fc ϵ RI (3) but is also associated with the $\gamma\delta$ TCR (4), the $\alpha\beta$ TCR (1, 2), DCAR (a novel C-type lectin immunoreceptor expressed on DC) (5, 6), mouse NKR1A/C/F (7), NKp46 (8), the high affinity IgG receptor Fc γ RI (9), the low affinity IgG receptor Fc γ RIIIa (10), and the IgA receptor Fc α RI (11, 12). Fc α RI, although a functional FcR, is a member of the leukocyte receptor cluster (LRC) whose genes are all encoded at 19q13.4 (13). A potentially charged TM

residue in the Ig superfamily receptors is generally indicative of assembly with a small signal transduction molecule (14). The activating LRC receptors, Fc α RI (11, 12, 15), the leukocyte Ig-like receptor A2 (LILR-A2, LIR7, ILT-1) (16), and the platelet collagen receptor glycoprotein VI (17, 18), NKp46 (8), and OSCAR (6), contain a TM arginine residue essential for interaction with FcR γ . Other than the TM arginine residue, it is not known if other TM residues of the activating LRC receptors are important in interacting with FcR γ . The TM regions of the FcRs, Fc ϵ RI, Fc γ RI, and Fc γ RIII, are also known to be important in assembly with FcR γ (19, 20) but are not homologous with the activating LRC members and lack the distinctive TM arginine residue. Therefore, the interaction of these two classes of receptors with FcR γ is fundamentally different. The ubiquitous expression of this activating subunit and its coupling with a number of receptors important in immunity and biology makes the molecular basis of the incorporation of this subunit into different receptors of wide interest.

This study defines novel transmembrane interactions of the FcR γ subunit with Fc α RI, an LRC member, and with the unrelated Fc ϵ RI. Although many of the interacting residues differ, some are common to both receptor classes and indicate a similar overall topology of interaction with FcR γ , which is probably indicative of all receptor interactions with FcR γ . Furthermore, the TM region of Fc α RI contains a leucine zipper-like sequence important in interactions with FcR γ , which is not apparent in the classical FcRs.

EXPERIMENTAL PROCEDURES

Antibodies—The polyclonal antisera, anti-FcR γ (3, 21) and anti-Fc α RI (22), and the anti-Fc α RI mAb A59 (23) have been described previously. Phycoerythrin-conjugated A59 was purchased from Pharmingen.

Construction and Mutagenesis of Receptor Expression Constructs—Restriction enzymes and DNA-modifying enzymes were all from New England Biolabs (Beverly, MA) except for PCR applications, which used the polymerase *Pwo* (Roche Applied Science). The DNA constructs WT human FcR γ chain in pMXI-EGFP (pBAR292) and Fc α RI in pMXpuro (pBAR252) were as described previously (21). Human Fc ϵ RI in pMXpuro (pBAR286) was constructed by releasing the Fc ϵ RI-encoding insert from pCK3 (24) with EcoRI and SalI, “blunt ending” with Klenow, and ligating into the SnaBI site of pMXpuro. The mutations Y8F, L14A, F15A, Y17F, I19A, and L21A in FcR γ and the mutations L215A, L217A, L220A, and L224A in Fc α RI were introduced by splice-overlap extension using standard molecular biology techniques or by QuickChange mutagenesis (Stratagene, La Jolla, CA). The Y25F and C26S mutant FcR γ has been described previously (21).

Transduction of IIA1.6 Cells for Receptor Expression—The murine B cell line IIA1.6 lacking endogenous Fc receptors (25) was transduced with recombinant retrovirus produced using the packaging line Phoenix (26) as described previously (21). Briefly, infection was with recombinant retrovirus expressing the WT or mutant FcR γ , and flow cytometry

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² The abbreviations used are: FcR γ , Fc ϵ RI γ subunit; FcR, Fc receptor; LRC, leukocyte receptor cluster; TM, transmembrane; mAb, monoclonal antibody; WT, wild type; EGFP, enhanced green fluorescent protein; FACS, fluorescence-activated cell sorter.

TABLE 1

TM region of FcR γ subunit and the CD3 ζ homologue

The aligned TM segments of the FcR γ subunit and the CD3 ζ chain with the residues responsible for dimerization of the TM segments are underlined. These include Cys-7 and Asp-11 and the residues belonging to the modified glycoporphin A dimerization motif (27). A heptad repeat pattern is characteristic of leucine zipper-like interactions. Residues a and d in the heptad repeat form the interaction interface. FcR γ residues mutated in this study and in Ref. 21 are marked with *. Residues important in the interaction with Fc α RI from mutagenesis data are shown in boldface type.

Name:	Putative TM:
heptad repeat	g a b c d e f g a b c d e f g a b c d e f
residue #	6 8 10 12 14 16 18 20 22 24 26
mutated this study	* * * * *
human FcR γ	<u>LCYILDA</u> <u>ILFLY</u> <u>GIVL</u> <u>TLLYC</u>
mouse FcR γ	<u>LCYILDA</u> <u>VLFLY</u> <u>GIVL</u> <u>TLLYC</u>
residue #	32 34 36 38 40 42 44 46 48 50
human CD3- ζ	<u>LCYLLD</u> <u>GILFI</u> <u>YGVIL</u> <u>TALFL</u>
mouse CD3- ζ	<u>LCYLLD</u> <u>GILFI</u> <u>YGVII</u> <u>TALYL</u>

was used to select for equivalent expression of the WT and mutant FcR γ cDNAs. FACS on EGFP for FcR γ expression was possible because EGFP was translated off the bicistronic FcR γ mRNA by means of an internal ribosome entry site. Next, FcR γ -expressing cells were infected with recombinant retrovirus containing Fc ϵ RI-pMXpuro (pBAR286) or Fc α RI-pMXpuro (pBAR252) and selected by puromycin treatment for transduced cells. Fc α RI-expressing cells were further selected by flow cytometry by staining with A59PE for receptor expression. Similarly, to investigate Fc α RI TM residues IIA1.6 cells expressing WT FcR γ were transduced and selected for expression of WT and mutant Fc α RI cDNAs.

FACS Analysis of Cells Expressing Fc Receptors—Cell surface expression of Fc ϵ RI α or Fc α RI was measured using mouse IgE myeloma TIB142 (ATCC, Manassas, VA) or phycoerythrin-conjugated mAb A59 Pharmingen, respectively, as described previously (21). Analysis of variance using a Dunnett multiple comparison test was performed with the program GraphPad Instat[®] (GraphPad Software Inc., San Diego, CA) and compared the levels of Fc ϵ RI expression in cells expressing mutant FcR γ subunits with the levels in cells expressing WT FcR γ subunit.

Immunoprecipitation of Receptors—Cell surface biotinylation with EZ-Link Sulfo-NHS-LC-Biotin (Pierce), lysis with 0.5% Brij-96 (Sigma), and immunoprecipitation with mAb A59 or rabbit anti-FcR γ antiserum and Western immunoblotting were performed as described previously (21).

Molecular Modeling—The template for modeling the FcR γ TM dimer (residues 6–26) and the first cytoplasmic residue Arg-27 was the NMR structure of the TM domain of glycoporphin A (Protein Data Bank code 1AFO). The glycoporphin A TM sequence was aligned with FcR γ (27), and residues of the template were changed to the corresponding residues of FcR γ . A rotamer search was performed to position side chains with the Insight II molecular modeling software (Accelrys, version 97.2). This initial template-based model was optimized with 5000 steps of conjugate-gradient energy minimization using the crystallography and NMR suite (CNS), version 1.0 (28). Stereochemistry and geometry of the final model were checked using the PROCHECK program (29).

RESULTS

Rationale for FcR γ TM Mutations—Transmembrane residues of FcR γ contribute both to its homodimerization and to its intermolecular interactions with other receptor subunits. FcR γ dimerization, like that of its homologue CD3 ζ , depends on an interchain disulfide and a modified glycoporphin A-like motif (Table 1) (27). Except for Ile-19, these

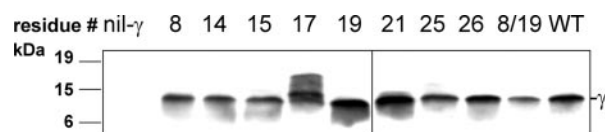


FIGURE 1. **Similar expression of WT and mutant FcR γ proteins in IIA1.6 cells.** IIA1.6 cells expressing WT or mutant FcR γ subunits were selected by FACS for EGFP expression, cultured, and lysed with 0.5% Brij-96. Immunoprecipitation, SDS-gel electrophoresis under reducing conditions, and subsequent probing of Western blots with anti-FcR γ antiserum confirmed similar expression levels. *nil-γ* refers to the parental IIA1.6 line.

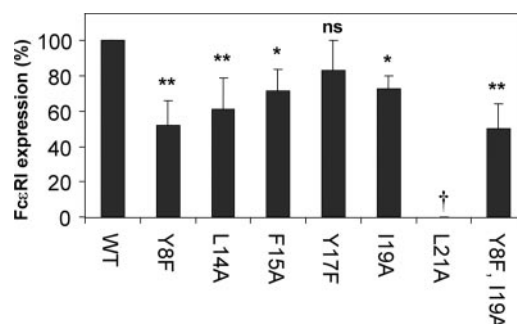


FIGURE 2. **FACS analysis of FcR γ -dependent surface expression of Fc ϵ RI.** IIA1.6 cells were stably transduced to express both the Fc ϵ RI (a ligand binding subunit) and the WT or mutant FcR γ subunits. Cells were selected by puromycin treatment for expression of the Fc ϵ RI cDNA, and these were tested for IgE binding using gating on the EGFP-positive population transduced for the FcR γ subunit. Fc ϵ RI expression is given as the percent of IgE binding (mean fluorescent intensity) relative to the WT receptor, and error bars indicate the standard deviation ($n = 5$). † indicates no IgE binding cells were detected in the Leu-21 mutant cells. In some of the mutant FcR γ -expressing cell lines, the levels Fc ϵ RI expression were highly significantly lower (**, $p < 0.01$), significantly lower (*, $p < 0.05$), or not significantly altered (ns, $p > 0.05$).

dimerization residues were not mutated, but a selection of other TM residues with polar or bulky side chains were mutated. These mutations were Y8F, L14A, F15A, Y17F, I19A, L21A, Y25F, and C26S. Thus, tyrosine residues were changed to phenylalanines, deleting the hydroxyl group, and other bulky hydrophobic residues were mutated to alanine. The transduced IIA1.6 cells were selected for FcR γ expression using flow cytometry to sort for EGFP, which is expressed via an internal ribosome entry site from the same bicistronic mRNA. The expression of the WT and mutant FcR γ proteins at similar levels was confirmed by immunoprecipitation with anti-FcR γ antiserum and Western blotting (Fig. 1). The FcR γ subunit is a small peptide, and some of the mutants display different mobility to the WT FcR γ subunit. The mutant Y17F is notably different having additional slower migrating species suggestive of some post-translational modification.

Fc γ /LRC TM Regions Bind a Common Site on the Fc ϵ R γ Chain

The Association of Fc γ TM Mutants with Fc ϵ RI—The cell surface expression of the high affinity IgE receptor is dependent on Fc γ associating with the ligand binding chain. Thus, surface expression of Fc ϵ RI, as measured by IgE binding to the transduced IIA1.6 cells, was used to assess the functional assembly of the Fc ϵ RI α chain with the TM residue mutants of Fc γ . There were three classes of Fc γ TM residues defined by this mutagenesis study. First, receptor cell surface expression was absolutely dependent on Leu-21 (Fig. 2), because no IgE binding activity, and hence no surface Fc ϵ RI, was found in WT Fc ϵ RI cells co-expressing L21A Fc γ . Second, a number of residues, Tyr-8, Leu-14, Phe-15, and Ile-19, had a comparatively modest effect on Fc ϵ RI expression. The most important of these was the Y8F mutation. Third, the mutation Y17A and the previously described mutations Y25F and C26S (21) did not decrease surface expression of Fc ϵ RI. The small differences in Fc ϵ RI

expression with the I19A mutant Fc γ was further tested using a Fc γ double mutant Y8F/I19A. This double mutant showed equivalent activity to that of the single Y8F mutant indicating that the Ile-19 makes at most only a minor contribution to association with Fc ϵ RI.

The Association of Fc γ TM Mutants with Fc α RI—Fc α RI is a member of the LRC, which can be expressed on the cell surface without association with the Fc γ chain (11, 12). Hence we assessed assembly of the Fc γ mutants with this receptor by surface biotinylating cells, immunoprecipitating the Fc γ subunit, and probing Western blots for co-precipitated biotinylated Fc α RI (Fig. 3). Two classes of TM residue were defined by these experiments. First, residues 14, 17, 21, 25, and 26 were all essential for the stability of the immunoprecipitated Fc α RI-Fc γ complex in Brij-96 detergent. A small amount of association with Fc α RI was found in the Fc γ Y25F immunoprecipitation. Second, mutation of residues 8, 15, 19, and 27 did not affect association of the Fc γ with Fc α RI. The double mutant Y8F/I19A showed some diminution of the amount of co-precipitated Fc α RI indicating that either these residues play a minor role in the interaction or that the dual mutation may compromise the conformational integrity of the interface of the TM dimer, because Ile-19 is part of the glycoporphin-A like motif.

A Model of Fc ϵ RI and Fc α RI Interaction with the Fc γ Subunit—To interpret the mutagenesis data, the TM regions of the disulfide-linked Fc γ dimer were modeled based on the structure of the glycoporphin A TM domain (Fig. 4). The two helices cross at a slight angle, and residues in the upper two-thirds of the TM region, including Asp-11, Gly-18, Ile-19, and Thr-22 in the variant glycoporphin motif, pack together along the central interface. Individual residues important in IgE receptor

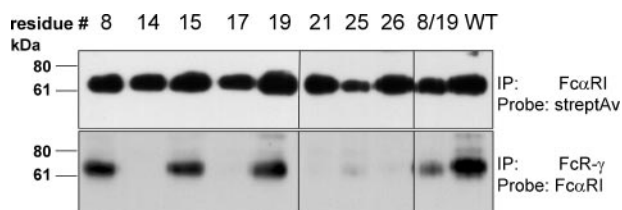


FIGURE 3. Fc γ TM residues affecting association with Fc α RI. IIA1.6 cells expressing Fc α RI and WT or mutant Fc γ subunits were surface-labeled with biotin and lysed with 0.5% Brij-96. Immunoprecipitation (IP) with anti-Fc α RI antiserum and probing with streptavidin-conjugated horseradish peroxidase showed equivalent Fc α RI expression and loading (top panel). Immunoprecipitation with anti-Fc γ antiserum and probing with anti-Fc α RI antiserum showed levels of Brij-96 stable receptor assembly (bottom panel).

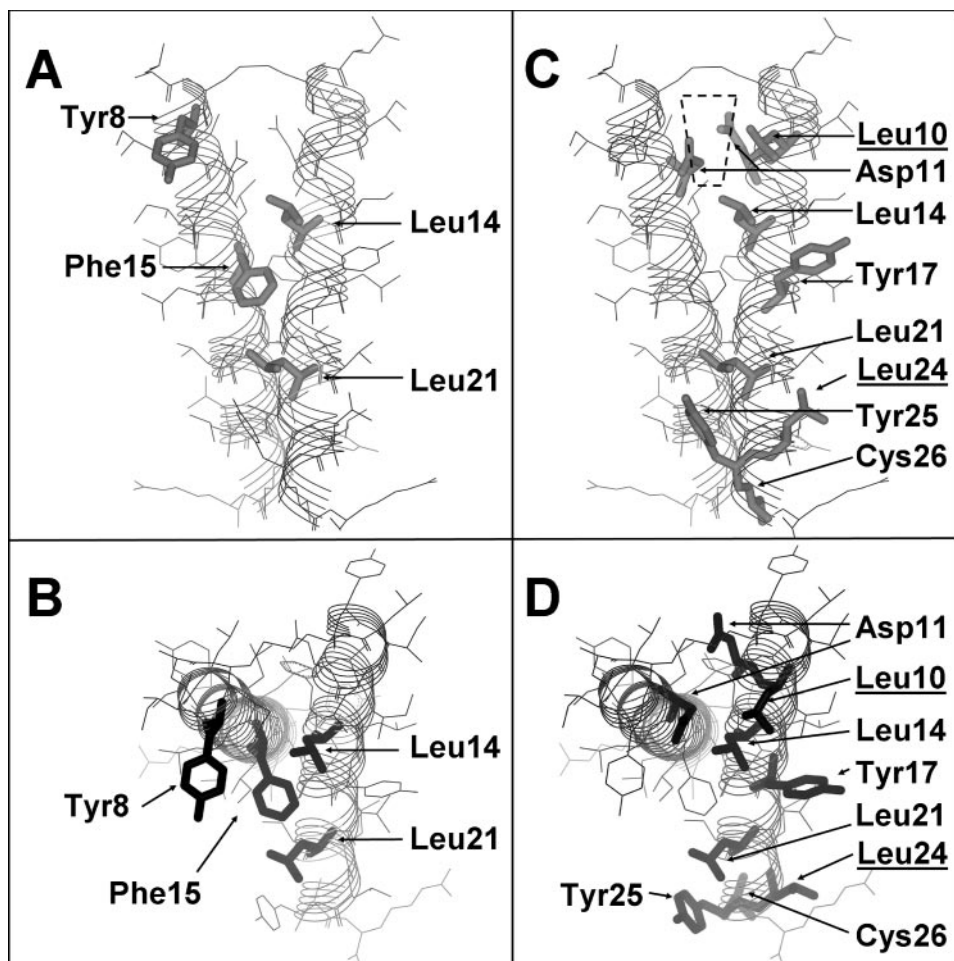


FIGURE 4. A model of the TM domains of the Fc γ dimer used to interpret mutagenesis data on receptor association. The Fc γ dimer model is shown with the α carbons as a ribbon representation and irrelevant side chains as thin lines. A and B, orthogonal views of the Fc γ dimer with residues defined by mutagenesis to be important in expression of Fc ϵ RI are shown as thick sticks. A, the view in the plane of the membrane; B, sights down the TM helices. C and D are equivalent views of the Fc γ dimer with residues important in stable assembly with Fc α RI represented as thick sticks. The residues Leu-10 and Leu-24 (underlined) are not defined by mutagenesis but inferred by the modeling and conform to the heptad repeat motif (see also Table 2). Asp-11 has been defined in other mutagenesis studies (reviewed in Ref. 41). The dashed V-shaped box indicates a channel through which Fc α RI Arg-209 may access one Asp-11. The figure was prepared using DS ViewerPro 6.0 (Accelrys, San Diego).

expression are displayed as *thick lines* on one side of the FcR γ dimer (Fig. 4, A and B). Collectively these residues form a single surface-accessible interface comprising two FcR γ chains and covering approximately two-thirds of the TM region, and a similar interface is duplicated on the opposite side of the FcR γ dimer. FcR γ residues Leu-14 and Leu-21 are on the same face of the helix and in the dimer lie along the cleft between the two helices. Residues Tyr-8 and Phe-15 are together on the opposite side from Leu-14 and Leu-21 in each helix, but in the dimer these are juxtaposed to lie along the cleft between the two helices. Ile-19 lies close to Leu-14 in the adjacent helix, and mutation indicates Ile-19 makes a small binding or structural contribution to association with Fc ϵ RI. A direct effect on receptor binding cannot be surmised over an indirect effect on conformation because Ile-19 contributes to the dimerization interface. This strongly suggests that the TM region of the Fc ϵ RI (α chain) interacts close to the interface between the two FcR γ helices as depicted (Fig. 4, A and B).

Residues important in interaction with Fc α RI are depicted in Fig. 4, C and D. It is noteworthy that carboxylate oxygens of residue Asp-11, which putatively interact with Arg-209 of Fc α RI, are accessible at the top of the FcR γ dimer where the crossing of the helices causes the inter-helical groove to widen forming a "V-shaped" channel capped by the interchain disulfide (Fig. 4C, *dashed box*). Residues Leu-14, Tyr-17, Leu-21, and Tyr-25, defined by mutagenesis in this study to be important in interacting with Fc α RI, all lie on one face of the FcR γ TM helix along the edge of the interface between the two helices and form a surface-accessible and almost linear site. The apparent lack of importance of Tyr-8 in the Fc α RI TM interaction suggests a different topology of interaction to that of the Fc ϵ RI TM. When taken with the known interaction of Fc α RI with Asp-11, the mutagenesis in this study has defined interacting residues on one face of the FcR γ helix that cover almost the full span of the membrane. Of these Leu-14, Tyr-17, and Leu-21 conform to a leucine zipper-like motif (Table 1). Also within this motif pattern are Leu-10 and Leu-24, which also may form part of the interface (*underlined* in Fig. 4, C and D). Together these data suggest that, like the Fc ϵ RI TM, the Fc α RI TM also associates at the interface between the two FcR γ TM regions but with a different hierarchy of importance of individual contacts (*e.g.* no Tyr-8) and therefore perhaps in a different topology to Fc ϵ RI. The mutation and modeling indicate the Fc α RI TM interacts via the interdigitation of bulky side chains in a leucine zipper-like interface.

The Association of Fc α RI TM Mutants with FcR γ —Next we looked for evidence of a complementary leucine zipper-like interface in the TM residues of Fc α RI. Because the well characterized putative charge interaction of Arg-209 with the FcR γ Asp-11 is in the upper TM region, we investigated the lower Fc α RI TM to avoid indirectly perturbing Arg-209. Hence, single alanine mutations of Fc α RI were made in the lower TM (*viz.* L215A, L217A, L220A, and L224A) to test if these affected interactions with FcR γ . Cells co-expressing WT or mutant Fc α RI with FcR γ were surface-biotinylated and immunoprecipitated with anti-FcR γ antiserum. In these immunoprecipitates detergent-stable association of WT-Fc α RI with FcR γ was strongly detected but was greatly diminished with all the Fc α RI mutants, indicating that this region of the TM contributes to interaction with FcR γ (Fig. 5). The level of associated L217A mutant was greatly reduced, and the L224A and L220A mutants of Fc α RI were even more weakly associated or undetectable. The most strongly associated mutant Fc α RI was L215A, but even so at a much lesser level than the WT. Residues Leu-217, Leu-224, and Leu-220, which were most affected by mutation, conform to a leucine zipper motif (Table 2) consistent with a reciprocal interface that interdigitates with that defined on FcR γ . It is noteworthy that the immunoprecipitated FcR γ

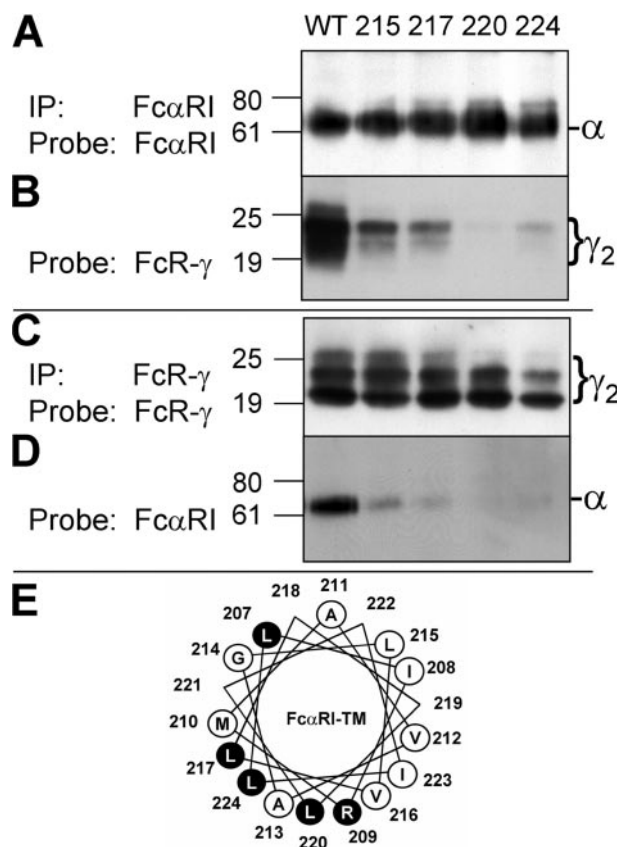


FIGURE 5. A leucine zipper interface is present in the TM of Fc α RI and conserved in the collagen receptor glycoprotein VI. A–D, IIA1.6 cells expressing FcR γ and WT or mutant Fc α RI subunits were lysed with 0.5% Brij-96 and immunoprecipitated (IP) and probed with anti-Fc α RI antiserum to show equivalent Fc α RI expression (A) or probed with anti-FcR γ antiserum demonstrating less stable association of the mutant receptors (B), or the lysate was immunoprecipitated and probed with anti-FcR γ antiserum to show equivalent FcR γ expression (C) or probed with anti-Fc α RI antiserum demonstrating less stable association of the mutant receptors (D). SDS-gel electrophoresis was under non-reducing conditions. E, helical wheel graphic (42) of the Fc α RI TM helix. TM residues of Fc α RI that are identical (*, see Table 2) or highly homologous (:) to residues of the activating LILR members are represented as *spheres* and of these residues those identical with platelet glycoprotein VI, the most distantly related TM region which nonetheless associates with FcR γ , are shown as *black-filled spheres* (see Table 2).

resolves as several bands on SDS-PAGE under nonreducing conditions (Fig. 5C) but as a single species under reducing conditions (Fig. 1). Furthermore, in our previous study (21) the C26S mutant FcR γ migrates largely as a single species irrespective of reducing or nonreducing conditions. This suggests a thiol-sensitive adduct at Cys-26, but this is in the main and not a palmitoyl adduct because labeled palmitate incorporates into FcR γ only at low stoichiometry (30).

DISCUSSION

The noncovalent association of TM regions of proteins occurs via interactions of polar residues and/or the steric matching of complementary shaped surfaces such as with the glycoporphin A motif (GXXXG motif) or by the interdigitation of bulky side chains analogous to the "knobs in holes" interactions of leucine zippers (31–33). The determinants of FcR γ chain TM interaction with FcRs and other immunoreceptors is only partially understood and is significant because of the widespread expression of this subunit and its promiscuous incorporation into a number of receptors. To address this we mutated selected polar or bulky FcR γ TM residues, excluding those putatively stabilizing the FcR γ homodimer. These experiments indicated that Fc α RI and Fc ϵ RI both interact with the interface between the two helices of the FcR γ dimer each using an overlapping set of FcR γ residues.

FcR/LRC TM Regions Bind a Common Site on the FcR γ Chain

TABLE 2

A comparison of TM regions of Fc α RI and other human LRC receptors and related mouse molecules

Blast was used to query the human genome BLAST page to identify LRC members related to Fc α RI. A selection of similar transmembrane regions to that of Fc α RI is shown in a ClustalW alignment made with high gap penalty of the Fc α RI. Identical (see *) residues are shown in boldface type. Note that for LILRA1 the sequence shown represents the C terminus of the protein and is without a stop transfer sequence. Note also the NKRP1 is a type II membrane glycoprotein so the TM sequence is reversed (C to N terminus) to give the outside to inside orientation.

Name:	Putative TM:	Accession:
Fc α RI residue #	209 217 220 224	
heptad repeat	a b c d e f g a b c d e f g a	
Fc alpha receptor	LIRMAVAGLVLVALLAILV	NP 579806
LILR molecules		
LILRB6; ILT8	LIRMGMAGLVFLVFLGILLF	NP_077294
LILRA2; LIR 7; ILT-1	LIRMGVAGLVLVVLGILLF	NP_006857
LILRA1; LIR6	LIRMGIAGLVFLVVLGILLF	NP_006854
LILRA4; ILT-7	LIRMGVAGLVLLFLGILLF	NP_036408
homology with Fc α RI:	***:..:*****: * : *	
Fc α RI equivalent residue #	209 217 220 224	
other LRC molecules + PIRA		
heptad repeat	a d a d a	
NCR 1; NK-p46	LIRMGLAFLVLVALVWFLV	NP_004820
OSCAR, isoforms 1,2,3	LVRLGLAGLVLSLGLVTF	NP_570127
PIR-A1	LIRMGMAVLVLIVLSILAT	NP_035217
Collagen receptor gpVI	LVRICLGAVILIILAGFLA	NP_057447
homology with Fc α RI:	*: *: .. : *: * : .	
Fc α RI equivalent residue #	209 217 220 224	
heptad repeat	a d a d a	
murine NKRP1A	LVRVLVSMGILTVVLLILGACSL	NP_034867
homology with Fc α RI:	*: *: *: : * .. : * **	
Fc α RI equivalent residue #	209 217 220 224	

This mutagenesis of FcR γ found residues Tyr-8, Leu-14, Phe-15, and Leu-21 interact with Fc α RI and influence receptor expression. These residues lie in the interface between the two helices in a model of the TM region of the FcR γ dimer. Leu-21 is clearly the most important interaction of those investigated with no surface expression of the FcR γ L21A IgE receptor complex detected. When the association of FcR γ mutants with Fc α RI was examined, Leu-14 and Leu-21 were again found to be important. For this receptor, however, other residues Tyr-17, Tyr-25, and Cys-26 also contributed to the affinity of the interaction with Fc α RI, although Tyr-8 did not, indicating both overlap and distinctiveness between the two receptor interactions with FcR γ . It should be noted that the tyrosine to phenylalanine mutations will affect polar interactions but may not affect a packing interaction dominated by the phenyl ring. When the mutagenesis data were interpreted by modeling the FcR γ dimer, it was apparent that the residues contributing to binding formed part of an extensive leucine zipper motif spanning the TM region (see heptad repeat sequence (Table 1) and Fig. 4, C and D).

Because we had defined novel residues in the TM region of FcR γ important in leucine zipper-like assembly with Fc α RI, we sought to define reciprocating residues in Fc α RI. Leu-217, Leu-220, and Leu-224 fit a heptad repeat suggesting they may make a leucine zipper-like interaction with FcR γ , and mutagenesis confirmed they contribute to the affinity of the interaction with FcR γ . That some effect was seen with the Fc α RI L215A mutant on the opposite face of the TM helix may suggest some higher order interactions contribute to the organization of the receptor:FcR γ assemblies. It is unlikely that the single leucine to alanine substitution would result in structural perturbation of the helical TM region. The existence of larger Fc α RI:FcR γ assemblies is

made more likely by the report that NKp46 forms a homodimer and that NKG2D and DAP10 form a hexameric complex (*i.e.* (NKG2D-DAP10-dimer)₂) (34).

Fc α RI and other receptors utilizing a TM arginine residue in interacting with FcR γ have significant sequence identities with Fc α RI-TM (Table 2). Although the LILR members have the greatest TM homology, the TM of platelet glycoprotein VI, an LRC member distantly related to Fc α RI but which nonetheless associates with FcR γ (17, 18), has five TM residues identical with Fc α RI (Fig. 5, *solid spheres*). Four of these identical residues conserve the Fc α RI interaction site for FcR γ comprising Arg-209 (11, 12) and the leucine zipper-like residues Leu-217, Leu-220, and Leu-224 (Fig. 5). NKp46, OSCAR, and PIRA also maintain the leucine zipper-like sequence, each with one amino acid replacement. Strikingly murine NKRP1A, an unrelated and type II orientated membrane protein, also has identities with Leu-217 and Leu-224 and a homology with Leu-220, thus possibly also using a zipper interaction with FcR γ .

The role of the TM dimer interface of FcR γ in receptor interactions is evident in other studies. First, CD3 ζ , a homologue of FcR γ , associates with the low affinity IgG receptor Fc γ RIIIa, an FcR closely related to Fc α RI. Human and mouse CD3 ζ TM regions differ in residue 46; this residue is equivalent to Leu-21 in FcR γ (Table 1). Human CD3 ζ (Leu-46) associates with Fc γ RIIIa in NK cells, whereas for the mouse CD3 ζ (Ile-46) the equivalent isoleucine residue, a steric isomer of leucine, is incompatible with Fc γ RIII association (35). Second, structural studies of the homologue CD3 ζ in TCR organization are informative (reviewed in Ref. 36). Mutagenesis and modeling of CD3 ζ TM to elucidate its topology in the murine TCR complex predicted that the CD3 δ / γ chain

bound to the cleft formed between the two CD3 ζ chains (27). Likewise, our study found the FcR/LRC chain bound at the cleft between the two Fc γ chains. Many CD3 ζ residues important in TCR assembly (Phe-40, Val-44, Ile-46, and Tyr-50 (27)) have equivalent residues in Fc γ (Phe-15, Ile-19, Leu-21, and Tyr-25), which participate in assembly with FcR and LRC subunits. Taken together these data indicate this site, comprising the interface between the CD3 ζ /Fc γ chains, is permissive for a number of different receptor interactions. Despite this, this site displays some fine selectivity as shown by Fc γ RIIIa association with the Leu-46 but not the Ile-46 forms of CD3 ζ (35).

Thus, apart from the well characterized Arg/Asp charge interaction, we identified a leucine zipper-like interaction between the TM regions of Fc α RI and Fc γ R. All LRC-related activating receptors and even the unrelated mouse NKRP1A/C/F are, given the homology of key TM residues, predicted to use this zipper interface in interactions with Fc γ R. Looking to the unrelated Fc receptors, the common requirement of Fc γ R Leu-14 and Leu-21 for interaction with both Fc α RI and Fc ϵ RI suggests the same interface between the helices of the Fc γ R dimer is universally where a receptor TM helix associates, although with varied contributions of different residues.

Finally, in accord with a universal interaction site on the Fc γ R dimer, Fc α RI and Fc γ RI have been shown in macrophage-like differentiated U937 cells to probably compete with each other for available Fc γ chain (37). Similarly eosinophils (38) express both Fc α RI, and LILRA2 (LIR7, ILT-1), mast cells express Fc ϵ RI and LILRA2 (39), and platelets express glycoprotein VI and Fc ϵ RI (40). Under such circumstances of expression of multiple receptors in the one cell, TM competition for association with the Fc γ subunit is likely to occur.

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