A Common Site of the Fc Receptor γ Subunit Interacts with the Unrelated Immunoreceptors FcαRI and FcεRI*

Received for publication, February 21, 2006, and in revised form, April 19, 2006. Published, JBC Papers in Press, April 19, 2006, DOI 10.1074/jbc.M601640200

Bruce D. Wines1, Halina M. Trist, Paul A. Ramsland, and P. Mark Hogarth

From the Helen Macpherson Smith Trust Infectious Disease Laboratory, The Macfarlane Burnet Institute for Medical Research and Public Health, Austin Health Campus, Heidelberg, Victoria 3084, Australia

The transmembrane (TM) region of the Fc receptor-γ (FcRγ) chain is responsible for the association of this ubiquitous signal transduction subunit with many immunoreceptor ligand binding chains, making FcRγ 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 FcRγ TM regions of the FcRγ expression of FcRγ high affinity IgE receptor FcεRI is a promiscuous receptor subunit originally identified as a subunit of the TCR (4), the phagocyte receptor CD16 (1), Leu-217, Leu-220, and Leu-224. The dependence of these two nonhomologous receptor interactions with FcRγ is fundamental. Likewise, detergent-stable FcRγ association with FcαRI 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 FcRγ dimer indicated these residues interacting with both FcαRI and FcεRI are near the interface between the two FcRγ TM helices. Furthermore, the FcRγ residues interacting with FcαRI form a leucine zipper-like interface with mutagenesis confirming a complementary interface comprising FcαRI residues Leu-217, Leu-220, and Leu-224. The dependence of these two nonhomologous receptor interactions on FcRγ Leu-14 and Leu-21 suggests that all the associated Fc receptors and the activating leucocyte 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 FcRγ subunit.

The Fc receptor γ subunit (FcRγ)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, γδ T cells, and CD4 αβ effector T cells (1, 2). It is a promiscuous receptor subunit originally identified as a subunit of the high affinity IgE receptor FcεRI (3) but is also associated with the γδ TCR (4), the αβ TCR (1, 2), DCAR (a novel C-type lectin immunoreceptor expressed on DC) (5, 6), mouse NKRP1A/C/F (7), NKp46 (8), the high affinity IgG receptor FcγRI (9), the low affinity IgG receptor FcγRIIa (10), and the IgA receptor FcαRII (11, 12). FcαRI, 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, FcαRI (11, 12, 15), the leukocyte Ig-like receptor A2 (LILR-A2, LIR7, ILT-T-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 FcRγ. Other than the TM arginine residue, it is not known if other TM residues of the activating LRC receptors are important in interacting with FcRγ. The TM regions of the FcRs, FcεRI, FcγRI, and FcγRIIa, are also known to be important in assembly with FcRγ (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 FcRγ 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 FcRγ subunit with FcαRI, an LRC member, and with the unrelated FcεRI. Although many of the interacting residues differ, some are common to both receptor classes and indicate a similar overall topology of interaction with FcRγ, which is probably indicative of all receptor interactions with FcRγ. Furthermore, the TM region of FcαRI contains a leucine zipper-like sequence important in interactions with FcRγ, which is not apparent in the classical FcRs.

EXPERIMENTAL PROCEDURES

Antibodies—The polyclonal antisera, anti-FcRγ (3, 21) and anti-FcαRI (22), and the anti-FcεRI 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 FcRγ chain in pMX-EGFP (pBAR292) and FcεRI in pMXpuro (pBAR252) were as described previously (21). Human FcεRI in pMX-puro (pBAR286) was constructed by releasing the FcεRI-encoding insert from pCK3 (24) with EcoRI and Sall, “blunt ending” with Klenow, and ligating into the SmAB I site of pMXpuro. The mutations Y8F, L14A, F15A, Y17F, I19A, and L21A in FcRγ and the mutations L215A, L217A, L220A, and L224A in FcαRI were introduced by splice-overlap extension using standard molecular biology techniques or by QuickChange mutagenesis (Stratagene, La Jolla, CA). The Y25F and C26S mutant FcRγ 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 FcRγ, and flow cytometry...
TABLE 1
TM region of FcRγ subunit and the CD3ζ homologue

| 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 g a b c d e f g a b c d e f g a b c d e f |
| residue #              | 9 10 12 14 16 18 20 22 24 26 |
| mutated this study     |                       |
| human FcRγ             | LCYI LDAILFLYGIVTLTLYC |
| mouse FcRγ             | LCYI LDAVFLYGIVTLTLYC  |
| residue #              | 32 34 36 38 40 42 44 46 48 50 |
| human CD3ζ             | LCYLLDGIIFYGVTALFL     |
| mouse CD3ζ             | LCYLLDGIIFYGVTALYL     |

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

FACS Analysis of Cells Expressing Fc Receptors—Cell surface expression of FcRRIa or FcRIR 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 FcRIR expression in cells expressing mutant FcRγ subunits with the levels in cells expressing WT FcRγ subunit.

Immuno precipitation 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-FcRγ antiserum and Western immunoblotting were performed as described previously (21).

Molecular Modeling—The template for modeling the FcRγ TM dimer (residues 6–26) and the first cytoplasmic residue Arg-27 was the NMR structure of the TM domain of glycophorin A (Protein Data Bank code 1AFO). The glycophorin A TM sequence was aligned with FcRγ (27), and residues of the template were changed to the corresponding residues of FcRγ. 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).

RESULTS

Rationale for FcRγ TM Mutations—Transmembrane residues of FcRγ contribute both to its homodimerization and to its intermolecular interactions with other receptor subunits. FcRγ dimerization, like that of its homologue CD3ζ, depends on an interchain disulfide and a modified glycophorin A-like motif (Table 1) (27). Except for Ile-19, these 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 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 FcRγ 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 FcRγ proteins at similar levels was confirmed by immunoprecipitation with anti-FcRγ antiserum and Western blotting (Fig. 1). The FcRγ subunit is a small peptide, and some of the mutants display different mobility to the WT FcRγ subunit. The mutant Y17F is notably different having additional slower migrating species suggestive of some post-translational modification.

FIGURE 1. Similar expression of WT and mutant FcRγ proteins in IIA1.6 cells. IIA1.6 cells expressing WT or mutant FcRγ 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-FcRγ antiserum confirmed similar expression levels. nil-γ refers to the parental IIA1.6 line.

FIGURE 2. FACS analysis of FcRγ-dependent surface expression of FcRIR. IIA1.6 cells were stably transduced to express both the FcRIR (a ligand binding subunit) and the WT or mutant FcRγ subunits. Cells were selected by puromycin treatment for expression of the FcRIR cDNA, and these were tested for IgE binding using gating on the EGFP-positive subunit. FcRIR expression was 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 FcRIR-expressing cell lines, the levels FcRIR expression were highly significantly lower (**, p < 0.01), significantly lower (*, p < 0.05), or not significantly altered (ns, p > 0.05).
**FcR/LRC TM Regions Bind a Common Site on the FcRγ Chain**

The Association of FcRγ TM Mutants with FceRI—The cell surface expression of the high affinity IgE receptor is dependent on FcRγ associating with the ligand binding chain. Thus, surface expression of FceRI, as measured by IgE binding to the transduced IIA1.6 cells, was used to assess the functional assembly of the FceRIα chain with the TM residue mutants of FcRγ. There were three classes of FcRγ 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 FceRI, was found in WT FceRI cells co-expressing L21A FcRγ. Second, a number of residues, Tyr-8, Leu-14, Phe-15, and Ile-19, had a comparatively modest effect on FceRI 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 FceRI. The small differences in FceRI expression with the I19A mutant FcRγ was further tested using a FcRγ 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 FceRI.

The Association of FcRγ TM Mutants with FcαRI—FcαRI is a member of the LRC, which can be expressed on the cell surface without association with the FcRγ chain (11, 12). Hence we assessed assembly of the FcRγ mutants with this receptor by surface biotinylating cells, immunoprecipitating the FcRγ subunit, and probing Western blots for co-precipitated biotinylated FcαRI (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 FcαRI-FcRγ complex in Brij-96 detergent. A small amount of association with FcαRI was found in the FcRγ Y25F immunoprecipitation. Second, mutation of residues 8, 15, 19, and 27 did not affect association of the FcRγ with FcαRI. The double mutant Y8F/I19A showed some diminution of the amount of co-precipitated FcαRI 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 glycophorin-A like motif.

A Model of FcεRI and FcαRI Interaction with the FcRγ Subunit—To interpret the mutagenesis data, the TM regions of the disulfide-linked FcRγ dimer were modeled based on the structure of the glycophorin 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 glycophorin motif, pack together along the central interface. Individual residues important in IgE receptor...
expression are displayed as thick lines on one side of the FcRγ dimer (Fig. 4, A and B). Collectively these residues form a single surface-accessible interface comprising two FcRγ chains and covering approximately two-thirds of the TM region, and a similar interface is duplicated on the opposite side of the FcRγ dimer. FcRγ 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 FcRγ. 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 FcRγ (α chain) interacts close to the interface between the two FcRγ helices as depicted (Fig. 4, A and B).

Residues important in interaction with FccRI 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 FccRI, are accessible at the top of the FcRγ 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 FccRI, all lie on one face of the FcRγ 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 FccRI TM interaction suggests a different topology of interaction to that of the FccRI TM. When taken with the known interaction of FccRI with Asp-11, the mutagenesis in this study has defined interacting residues on one face of the FcRγ 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 FccRI TM, the FccRI TM also associates at the interface between the two FcRγ 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 FccRI. The mutation and modeling indicate the FccRI TM interacts via the interdigitation of bulky side chains in a leucine zipper-like interface.

The Association of FccRI TM Mutants with FcRγ—Next we looked for evidence of a complementary leucine zipper-like interface in the TM residues of FccRI. Because the well characterized putative charge interaction of Arg-209 with the FcRγ Asp-11 is in the upper TM region, we investigated the lower FccRI TM to avoid indirectly perturbing Arg-209. Hence, single alanine mutations of FccRI were made in the lower TM (viz. L215A, L217A, L220A, and L224A) to test if these affected interactions with FcRγ. Cells co-expressing WT or mutant FccRI with FcRγ were surface-biotinylated and immunoprecipitated with anti-FcRγ antiserum demonstrating less stable association of the mutant receptors (B), and the lysate was immunoprecipitated and probed with anti-FcRγ antiserum to show equivalent FcRγ expression (C) or probed with anti-FccRI antiserum demonstrating less stable association of the mutant receptors (D). SDS-gel electrophoresis was under nonreducing conditions. E, helical wheel graphic (42) of the FccRI TM helix. TM residues of FccRI that are identical (*, see Table 2) or highly homologous (i) 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 FcRγ, 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 FcRγ 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 FcRγ 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 (GXXG motif) or by the interdigitation of bulky side chains analogous to the “knobs in holes” interactions of leucine zippers (31–33). The determinants of FcRγ chain TM interaction with FccRs 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 FcRγ TM residues, excluding those putatively stabilizing the FcRγ homodimer. These experiments indicated that FccRI and FccRI both interact with the interface between the two helices of the FcRγ dimer each using an overlapping set of FcRγ residues.

FIGURE 5. A leucine zipper interface is present in the TM of FccRI and conserved in the collagen receptor glycoprotein VI. A–D, IIA1.6 cells expressing FcRγ and WT or mutant FccRI subunits were lysed with 0.5% Brij-96 and immunoprecipitated (IP) and probed with anti-FccRI antiseraum to show equivalent FcRγ expression (A) or probed with anti-FcRγ antiseraum demonstrating a stable association of the mutant receptors (B), or the lysate was immunoprecipitated and probed with anti-FcRγ antiserum to show equivalent FcRγ expression (C) or probed with anti-FccRI antiseraum demonstrating a stable association of the mutant receptors (D). S-D-gel electrophoresis was under nonreducing conditions. E, helical wheel graphic (42) of the FccRI TM helix. TM residues of FccRI that are identical (*, see Table 2) or highly homologous (i) 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 FcRγ, are shown as black-filled spheres (see Table 2).
### TABLE 2
A comparison of TM regions of FcαRI and other human LRC receptors and related mouse molecules

| Name: | Putative TM: | Accession: |
|-------|--------------|------------|
| FcαRI residue # | 209 217 220 224 | |
| heptad repeat | abcdefgabcdefg | |
| Fc α receptor | LIRMAVAGLVLVALLAILV | NP_579806 |
| **LILR molecules** | | |
| LILRB6; ILT8 | LIRMGAMGLVLVFGLILLF | NP_077294 |
| LILRA2; LIR 7; ILT-1 | LIRMGAVGLVLVGGLLF | NP_006857 |
| LILRA1; LIR6 | LIRMGIAGVLVLGGLLF | NP_006854 |
| LILRA4; ILT-7 | LIRMUGAVGLVLFGGLLF | NP_036408 |
| homology with FcαRI: | ***:****: | *:* |
| FcαRI equivalent residue # | 209 217 220 224 | |
| **other LRC molecules + PIRA** | | |
| heptad repeat | a da a a | |
| NCR 1; NK-46 | LIRMGIAFLVGLVWFLV | NP_004820 |
| OSCAR, isoforms 1,2,3 | LVRGLAGLVLISLAGLVTF | NP_570127 |
| PIR-A1 | LIRMGAMGLVLVSLAT | NP_035217 |
| Collagen receptor gpVI | LVRICGAVILLAGFLA | NP_057447 |
| homology with FcαRI: | **:**:**:**:**::*: | |
| FcαRI equivalent residue # | 209 217 220 224 | |
| heptad repeat | a da a a | |
| murine NKRP1A | LVRVLVSMGLITVVLILGACSL | NP_034867 |
| homology with FcαRI: | **:**:**:**:**:**:** | |
| FcαRI equivalent residue # | 209 217 220 224 | |

This mutagenesis of FcRγ found residues Tyr-8, Leu-14, Phe-15, and Leu-21 interact with FcαRI and influence receptor expression. These residues lie in the interface between the two helices in a model of the TM region of the FcRγ dimer. Leu-21 is clearly the most important interaction of those investigated with no surface expression of the FcRγ L21A IgE receptor complex detected. When the association of FcαRI mutants with FcαRI 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 FcαRI, although Tyr-8 did not, indicating both overlap and distinctiveness between the two receptor interactions with FcRγ. 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 FcRγ 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. 2, C, and D).

Because we had defined novel residues in the TM region of FcRγ important in leucine zipper-like assembly with FcαRI, we sought to define reciprocating residues in FcαRI. Leu-217, Leu-220, and Leu-224 fit a heptad repeat suggesting they may make a leucine zipper-like interaction with FcRγ, and mutagenesis confirmed they contribute to the affinity of the interaction with FcRγ. That some higher order interactions contribute to the organization of the receptor:FcRγ 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)) (34).

FcαRI and other receptors utilizing a TM arginine residue in interacting with FcRγ have significant sequence identities with FcαRI-TM (Table 2). Although the LILR members have the greatest TM homology, the TM of platelet glycoprotein VI, an LRC member distantly related to FcαRI but which nonetheless associates with FcRγ (17, 18), has five TM residues identical with FcαRI (Fig. 5, solid spheres). Four of these identical residues conserve the FcαRI interaction site for FcRγ 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 FcRγ.

The role of the TM dimer interface of FcRγ in receptor interactions is evident in other studies. First, CD3ζ, a homologue of Fcγ, associates with the low affinity IgG receptor FcγRIIA, an FcR closely related to FcαRI. Human and mouse CD3ζ TM regions differ in residue 46; this residue is equivalent to Leu-21 in FcαRI (Fig. 5, solid spheres). Four of these identical residues conserve the FcαRI interaction site for Fcγ 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 Fcγ.
bound to the cleft formed between the two CD3ζ chains (27). Likewise, our study found the FcR/LRC chain bound at the cleft between the two FcRγ chains. Many CD3ζ residues important in TCR assembly (Phe-40, Val-44, Ile-46, and Tyr-50 (27)) have equivalent residues in FcRγ (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 CD3ζ/FcRγ chains, is permissive for a number of different receptor interactions. Despite this, such site displays some fine selectivity as shown by FcyRIIα association with the Leu-46 but not the Ile-46 forms of CD3ζ (35).

Thus, apart from the well characterized Arg/Asp charge interaction, we identified a leucine zipper-like interaction between the TM regions of FcRRI and FcRγ. 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 FcRγ. Looking to the unrelated Fc receptors, the common requirement of FcRγ-Leu-14 and Leu-21 for interaction with both FcRRI and FcRRII suggests the same interface between the helices of the FcRγ 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 FcRγ dimer, FcRRI and FcRγ have been shown in macrophage-like differentiated U937 cells to probably compete with each other for available FcRγ chain (37). Similarly eosinophils (38) express both FcRRI and FcRRII, suggesting the same interface between the helices of the FcRRI chain (37).

### Acknowledgments

We thank Dr. Cees van Kooten for anti-FcR/LRC antibody and Drs. A. A. M. van den Boogaard, M. T. E. Sturk, and P. J. E. van der Voort for helpful discussions. We thank the FcγRIIb patient, Z. H. H., for his participation in the study. This work was supported by grants from the Netherlands Cancer Society, the Dutch Arthritis Foundation, and the Dutch Fyssen Foundation.

### References

1. Pfefferkorn, L. C., and Yeaman, G. R. (1994) J. Biol. Chem. 270, 29781–29787
2. Volz, A., Wende, H., Laun, K., and Ziegler, A. (2001) Immunol. Rev. 181, 39–51
3. Coop, S., Lankford, S. P., Bonifacino, J. S., and Klausner, R. D. (1991) Nature 351, 414–416
4. Bakker, J. E., de Haaij, S., den Hartog-Jager, C. F., Bakker, J., Viddas, G., van Edmond, M., van de Winkel, J. G., and Leusen, J. H. (2006) J. Immunol. 176, 3607–3610
5. Nakajima, H., Marianidis, J., Angman, L., and Colonna, M. (1999) J. Immunol. 162, 5–8
6. Tsuji, M., Esumi, Y., Arai, M., and Takayama, H. (1997) J. Biol. Chem. 272, 3528–3531
7. Clemot, J. M., Polgar, J., Magnenat, E., Wells, T. N., and Clemot, K. J. (1999) J. Biol. Chem. 274, 29019–29024
8. Patel, P. T., Bjorkkaug, L., Hutchinson, M. J., and Allen, J. M. (1995) Mol. Membr. Biol. 12, 309–312
9. Kim, M. K., Huang, Z. Y., Hwang, P. H., Jones, B. A., Sato, N., Hunter, S., Kim, H., Worth, R. G., Indik, Z. K., and Rechberg, A. D. (2003) Blood 101, 4479–4484
10. Wines, B. D., Trist, H. M., Monteiro, R. C., Van Kooten, C., and Hogarth, P. M. (2004) J. Biol. Chem. 279, 26339–26345
11. de Boog, P. J., van Zandbergen, G., De Fijter, J. W., Klar-Mohamed, N., van Seggelen, A., Brandtzaeg, P., Daha, M. R., and Van Kooten, C. (2002) J. Immunol. 168, 1252–1258
12. Monteiro, R. C., Cooper, M. D., and Kubagawa, H. (1992) J. Immunol. 148, 1764–1770
13. Hulett, M. D., McKenzie, I. F., and Hogarth, P. M. (1993) Eur. J. Immunol. 23, 640–645
14. Moretta, A., Bottino, C., Vitale, M., Pende, D., Cantoni, C., Moretta, L., and Moretta, A. (1991) J. Immunol. 147, 187–189
15. Cosson, P., Lankford, S. P., Bonifacino, J. S., and Klausner, R. D. (1991) J. Exp. Med. 173, 2212–2218
16. Voss, T. H., Indik, Z. K., and Schreiber, A. D. (1998) J. Immunol. 160, 5–8
17. Montalvan, M., and Capron, A., and Capron, M. (2000) J. Immunol. 165, 2212–2218