Crystal Structure of the Ectodomain of Human FcRI*

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Human FcRI (CD89) is the receptor specific for IgA, an immunoglobulin that is abundant in mucosa and is also found in high concentrations in serum. Although FcRI is an immunoglobulin Fc receptor (FcR), it differs in many ways from FcRs for other immunoglobulin classes. The genes of most FcRs are located on chromosome 1 at 1q21–23, whereas FcRI is on chromosome 19, at 19q13.4, a region called the leukocyte receptor complex, because it is clustered with several leukocyte receptor families including killer cell inhibitory receptors (KIRs) and leukocyte Ig-like receptors (LIRs). The amino acid sequence of FcRI shares only 20% homology with other FcRs but it has around 35% homology with its neighboring LIRs and KIRs. In this work, we analyzed the crystal structure of the ectodomain of FcRI and examined structure similarities between FcRI and KIR2DL1, KIR2DL2 and LIR-1. Our data show that FcRI, KIRs, and LIRs share a common hydrophobic core in their interdomain interface, and FcRI is evolutionally closer to LIR than KIR.

In humans, IgA is the most abundant immunoglobulin in secretions, and it constitutes about 20% of the immunoglobulin pool in serum (1, 2). Its turnover rate is faster than other immunoglobulins, the daily production of IgA exceeds all other immunoglobulins combined (3). Undoubtedly, IgA should to play important roles in immune defense against invaded pathogens.

Five types of IgA receptors have been recognized so far. They are FcRI (CD89), the polymeric Ig receptor, FcαR, the transferrin receptor, and the asialo IgG protein receptor (1). Among them, FcRI is the only one that specifically binds IgA. On ligation of IgA complexed with antigens, FcRI is able to mediate various cellular responses including phagocytosis, antibody-dependent cell cytotoxicity, oxidative bursts, and release of inflammatory mediators (1).

FcRI belongs to the immunoglobulin superfamily and contains an extracellular region of 206 amino acids, a transmembrane domain of 19 amino acids and a cytoplasmic region of 41 amino acids (4). The extracellular region of FcRI consists of two Ig-like domains, EC1 and EC2, and six potential sites for N-glycosylation. The receptor binds IgA1 and IgA2 with an equal affinity (5). A number of residues including Tyr285, Arg52, Tyr81, Arg82, Ile83, Gly84, His85, and Tyr86 on FcRI are potentially involved in IgA binding (6, 7).

Although FcRI is an immunoglobulin Fc receptor (FcR), it differs in many ways with FcRs for other immunoglobulin classes. IgG receptor FcγRIII and IgE receptor FcεRI bind antibodies in the near hinge regions and form 1:1 complexes (8, 9), whereas FcRI binds the C1q-C2-C3 interface of Fcα (10, 11) and preferably forms 2:1 complex with a single Fcα homodimer (12). It has been reported that FcγRs and FcεRI use their membrane proximal-domain and linker region binds immunoglobulin (8, 9, 13, 14), whereas FcRI uses its membrane-distal domain EC1 to bind IgA (15).

The genes of most FcRs are located in chromosome 1 at 1q21–23 (16), whereas FcRI is in chromosome 19, at 19q13.4 (17, 18), a region called the leukocyte receptor complex because it is clustered with several leukocyte receptor families including killer cell inhibitory receptors (KIRs) and leukocyte Ig-like receptors (LIR/LILR/ILTs) (17, 18). The amino acid sequence of FcRI shares only 20% homology with other FcRs, but it has around 35% homology with its neighboring LIRs and KIRs (1).

In this paper, we report our analysis of the crystal structure of the ectodomain of FcRI expressed in Escherichia coli and its comparison with FcRs, LIR, and KIR.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The construction and expression of the extracellular ligand binding domain of a human FcRI will be described in detail elsewhere. Briefly, residues 1–207 of the mature sequence were subcloned into a Novagen pET-28a vector using the NcoI and Xhol restriction sites and an E. coli BL21(DE3) strain. Two additional amino acids (Met-Ala) were added to the 5′ end of the gene, and a histidine tag (His6) was added to the 3′ end to facilitate the expression and purification. The protein was first expressed in an inclusion body form and then reconstituted in vitro. The isolation of the inclusion bodies was started with an intense combined lysosome/sonification procedure to open virtually all cells. Subsequent washing steps with Triton X-100 and NaCl yielded a product with a purity of >80% as estimated by SDS-PAGE. The

* This work was supported by grants from the National Natural Science Foundation of China (No. C0302050102), Natural Science Foundation of Beijing (No. 7012026), Project “863” (No. 2001AA233011) and Project “973” (No. G1999075600 and No. 200213A711A12). The authors also thank the Ministry of Education, the Chinese Academy of Science, the Chinese Academy of Medical Sciences for financial support.

† The abbreviations used are: FcR, Fc receptor; KIR, killer cell inhibitory receptor; LIR, leukocyte Ig-like receptor; r.m.s.d., root mean square deviation.

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Crystallization, Data Collection, and Structure Determination—The FcRRI crystals were obtained by the hanging-drop method. The crystals were grown from a buffer of 11.4% polyethylene glycol-8000 in 100 mM sodium Hepes buffer, pH 7.6, containing 5% (v/v) ethanol glycol, 3% (v/v) 2-Mercaptoethanol, 0.5 M ammonium acetate, and 15% (v/v) protein concentration of ~12 mg/ml. The Se-Met derivative crystal was grown from the same conditions. The Se-Met derivative data were collected at the Spring8 beamline BL41XU under 100 K at wavelengths 0.9798 Å, and contained 191 residues of FcRI (MA), C-terminal 15 residues (DSIHQDYTTQNLILE), and residues Asn165, and Val166, respectively.

Building were done using the program O (22), version 8.0. Refinement could be built. The initial chain tracing and all subsequent model except for one flexible loop and several residues at the termini resultative electron density map was of sufficient quality that the entire model was initially refined as a rigid body with data in the resolution range 8.0–4.0 Å resolution. The resolution was extended gradually, and substructure determination was performed using CNS1.0 (23) and merged synchrotron data with \( F_{obs} > 0 \). The Bijvoet pairs of the data used in refinement are unmerged. The model was initially refined as a rigid body with data in the resolution range 0.0–4.0 Å resolution. The resolution was extended gradually, and subsequent refinement used protocols including anisotropic temperature factor refinement, energy minimization, and slow cooled simulated annealing. Several rounds of manual refitting using omit maps permitted the missing loop regions to be traced and side chains built. 68 water molecules were built into the electron density when a \( F_{o} - F_{c} \) map, contoured at 3.5σ, coincided with well defined electron density of a 2\( F_{o} - F_{c} \) map contoured at 1σ. The N-terminal 2 additional residues (MA), C-terminal 15 residues (DSIHQDYTTQNLILE), and residues 56–59 (FWNE) were disordered in the crystal. The final model contained 191 residues of FcRRI and 68 solvent molecules. \( R_{	ext{merge}} \) and \( R_{	ext{free}} \) were 0.210 and 0.239, respectively, for data in the resolution range 40.0–21 Å. The structure contains two cis prolines at position 154 and 161. None of the main-chain torsion angles are located in disallowed regions of the Ramachandran plot. Statistics for data collection, phasing, and refinement are shown in Table I.

For analyses of interdomain angles, contacts, and buried surface areas, D1 was defined as residues 1–100 and D2 was defined as residues 101–195, following the structure-based definition of KIR2DL1 domain boundaries (24). Interdomain contacts were defined as being within 3.6 Å of the partner domain and identified using CONTACT (35). Buried surface areas were calculated using SURFACE (36) with a 1.4 Å probe radius.

RESULTS AND DISCUSSION

The crystal structure of the extracellular region of FcRRI consists of two Ig-like domains, EC1 (residues Gln1 to Glu100) and EC2 (residues Pro105 to His199) (Fig. 1a). EC1 and EC2 obey the typical heart-shaped arrangement, and a short linker (Leu101 to Lys194) connects them together. Both domains are primarily composed of \( \beta \)-structure arranged into two antiparallel \( \beta \) sheets with a KIR-like folding topology. The sheets are closely packed against each other with the conserved disulfide bridge connecting the strands B and F on the opposing sheets. Three \( \beta \)-strands are found in N termini of EC1 (Glu to Asp4), EF loops of EC1 (Ala71, Asn72, and Lys73) and EC2 (Leu164, Asn165, and Val166), respectively.

In the EC1 domain, four anti-parallel \( \beta \) strands (A, B, E, D) oppose a sheet of five \( \beta \) strands (C', C, F, G, A') (Fig. 1b). There is a \( \beta \)-bulge (Ser31 to Thr38) in the G strand of EC1 splitting the strand into two short \( \beta \) strands.

The EC2 domain is built up from eight \( \beta \) strands arranged such that three strands (A, B, E) form one \( \beta \)-sheet and five strands (C', C, F, G, A') form a second \( \beta \)-sheet. EC2 does not have a strand in the corresponding position to strand D of EC1.
D1 and D2 domains of KIR and LIR, the Ca atoms of these three receptors were superimposed to analyze their structural similarities. As shown in Fig. 2a, the overall structures of the three receptors are similar especially for the EC2 and D2 domains. The major difference is found in the corresponding position of EC1 C, C', and D strands of FcαRI. In LIR-1 D1, strands C' and D are replaced by two 3₁₀ helices. On the other hand, the C' strand of FcαRI is shorter than that of KIR2DL1
and KIR2DL2. Hence, the C-C'/H11032 loop in Fc'H9251 RI EC1 forms earlier (Fig. 2b), allowing the C-C'/H11032 loop and F-G loop to adopt a clamp-like arrangement. A similar feature can also be found in many other FcR structures. The Ca traces of FcRI, FcR, FcγRIa, FcγRIib, and FcγRIII are colored purple, blue, orange, green, and red, respectively.

The interdomain angle of FcRI is closer to that of LIR-1 (84 to 90°) (25), but larger than that of KIR2DL2 (60 to 80°) (26). The hydrophobic core interface observed in FcHR also exists in LIR-1 and KIR2DL2 (25, 26). Amino acid sequence alignment shows the 12 hydrophobic residues, especially Tyr181 and Trp183, which play an important role in stabilizing the inter-domain angle in Fc'R are also conserved in LIR-1 and KIRs, having only one residue (Leu101→Ala) different for LIR-1, three residues (Val17→Leu, Val98→Ile, and Tyr173→Phe) different for KIR2DL2 and four residues (Val17→Leu, Val98→Ile, Thr99→Ile and Tyr173→Phe) different for KIR2DL1. These 12 residues are also conserved in a KIR from cow, with only one residue (Tyr102→Ser) different from Fc'H9251 RI in this region (Fig. 3). Moreover, a bovine IgG2 FcR, Fc'R2, also possesses most of these hydrophobic residues and only four

![Diagram](http://www.jbc.org/Downloaded from http://www.jbc.org/)
show that FcRI is a member of the leukocyte receptor complex and evolutionarily closer to LIR than KIR. All members of this complex found so far share a common hydrophobic core structure. The crystal structure also locates the residues that are involved in FcRI binding to IgA.

Acknowledgments—We thank Fei Sun, Feng Xu, and Zhiyong Lou for assistance with data collection at Spring-8 BEAMLINE BL41XU. We also thank Drs. Mark Bartlam and Yiwei Liu for valuable discussion and reading of the manuscript.

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J. Biol. Chem. 2003, 278:27966-27970.
doi: 10.1074/jbc.C300223200 originally published online June 3, 2003

Access the most updated version of this article at doi: 10.1074/jbc.C300223200

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