The Structure of a Human Type III Fcγ Receptor in Complex with Fc*

Sergei Radaev‡, Shawn Motyka‡, Wolf-Herman Fridman¶, Catherine Sautes-Fridman§, and Peter D. Sun‡

From the ²Structural Biology Section, Laboratory of Immunogenetics, NIAID, National Institutes of Health, Rockville, Maryland 20852; the ³Biochemistry, Cellular, and Molecular Biology Program, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205; and the ¶Laboratory of Cellular and Clinical Immunology, INSERM Unit 255, Curie Institute, 75005 Paris, France

Fcγ receptors mediate antibody-dependent inflammatory responses and cytotoxicity as well as certain autoimmune dysfunctions. Here we report the crystal structure of a human Fc receptor (FcγRIIIb) in complex with an Fc fragment of human IgG1 determined from orthorhombic and hexagonal crystal forms at 3.0- and 3.5-Å resolution, respectively. The refined structures from the two crystal forms are nearly identical with no significant discrepancies between the coordinates. Regions of the C-terminal domain of FcγRIII, including the BC, Cε, FG loops, and the Cε β-strand, bind asymmetrically to the lower hinge region, residues Leu234-Pro238, of both Fc chains creating a 1:1 receptor-ligand stoichiometry. Minor conformational changes are observed in both the receptor and Fc upon complex formation. Hydrophobic residues, hydrogen bonds, and salt bridges are distributed throughout the receptor-Fc interface. Sequence comparisons of the receptor-ligand interface residues suggest a conserved binding mode common to all members of immunoglobulin-like Fc receptors. Structural comparison between FcγRIII-Fc and FcεRI-Fc complexes highlights the differences in ligand recognition between the high and low affinity receptors. Although not in direct contact with the receptor, the carbohydrate attached to the conserved glycosylation residue Asn297 on Fc may stabilize the conformation of the receptor-binding epitope on Fc. An antibody-FcγRIII model suggests two possible ligand-induced receptor aggregations.

Fcγ receptors, which are expressed on the majority of hematopoietic cells, play important roles in antibody-mediated immune responses. The binding of antigen-bound immunoglobulins (Ig) to Fc receptors activates their effector functions and leads to phagocytosis, endocytosis of IgG-opsonized particles, as well as antibody-dependent cellular cytotoxicity. The three major types of Fc receptors are Fcγ, Fcε, and neonatal Fc receptors. Except for the neonatal Fc receptor and FcεRI (CD23), which are related structurally to class I major histocompatibility antigens and C-type lectins, respectively, all other known Fc receptors are members of the immunoglobulin superfamily (1, 2). Among them, FcγRI and FcεRI are high affinity Fc receptors for IgG and IgE, respectively, with dissociation constants ranging from 10⁻⁸ to 10⁻¹² M. All other receptors for IgG, such as FcγRII and FcγRIII, are low affinity receptors with dissociation constants ranging from 10⁻⁵ to 10⁻⁷ M (3–5). In addition to variations in affinity, each receptor displays distinct IgG subtype specificities. Unlike the high affinity receptors that can bind monomeric antibodies, the low affinity receptors preferentially bind to and are activated by immune complexes.

Human FcγRIII exists as two isoforms, FcγRIIA and FcγRIIB, that share 96% sequence identity in their extracellular immunoglobulin-binding regions. FcγRIIA is expressed on macrophages, mast cells, and natural killer cells as a transmembrane receptor. In contrast, FcγRIIB, present exclusively on neutrophils, is anchored by a glycosyl-phosphatidylinositol linker to the plasma membrane. Although FcγRIIA associates with the immunoreceptor tyrosine-based activation motif containing FcεRI γ-chain or the T cell receptor ζ-chain for its signaling, FcγRIIB lacks a signaling component. Nevertheless, it plays an active role in triggering Ca²⁺ mobilization and in neutrophil degranulation (6, 7). In addition, FcγRIIB, in conjunction with FcγRIIA, activates phagocytosis, degranulation, and the oxidative burst that leads to the clearance of opsonized pathogens by neutrophils. A soluble form of FcγRIIB was reported to activate the CR3 complement receptor-dependent inflammatory process (8).

The Fc binding region on FcγRII and FcγRIII has been identified through the work of chimeric receptors with FcεRI as primarily the membrane proximal domain, including both the BC and FG loops. Further site-directed mutations have revealed several residues of the receptor critical to Fc binding (9–11). Similar regions on the ε-chain of FcεRI were also identified to be critical for IgE binding affinity (12). The receptor binding site on Fc has been located through the construction of chimeric IgG molecules and mutational analysis at the lower hinge region, residues located in the hinge region between the Cα1 and Cα2 domains and immediately adjacent to the N terminus of the Cα2 domain of IgG (13–15). In particular, residues 234–238 (Leu-Leu-Gly-Gly-Pro) of the lower hinge of IgG1 have been implicated in the receptor binding. The corresponding region of IgE has also been implicated in the FcεRI binding (16). Apart from the lower hinge region, a few residues on the Cα2 domain of an IgG2b were also suggested to interact

* This work was supported by the intramural research funding of NIAID, National Institutes of Health and by INSERM, Institut Curie, France. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Structural Biology Section, Laboratory of Immunogenetics, NIAID, National Institutes of Health, 12441 Parklawn Dr., Rockville, MD 20852. Tel.: 301-496-3230; Fax: 301-402-0284; E-mail: psun@nih.gov

‡ The abbreviations used are: FcγRI and FcεRI, Fc receptors for IgG and IgE of the immunoglobulin superfamily; r.m.s., root mean square; FcRn, neonatal Fc receptor; RF, rheumatoid factors.
with the receptor (17). However, with the exception of the neonatal Fc receptor, the molecular recognition between the Fc receptors and Fc are to be elucidated (18).

The recent crystal structures of FcRIα, FcRIIA, and FcRIIB have each revealed a conserved Ig-like structure, with particularly the small hinge angle between the two Ig-like domains, which is unique to the Fc receptors (19–21). We report here the crystal structure of a human FcRIII in complex with the Fc portion of a human IgG1 determined from two crystal forms.

**EXPERIMENTAL PROCEDURES**

**Protein Expression, Purification, and Crystallization**—The extracellular part of the human FcRIII receptor, residues 1–172, was expressed as *Escherichia coli* inclusion bodies and then reconstituted in *vitro* as described previously (22). Fc fragments of human IgG1 antibody were prepared by the papain digestion as previously reported (23, 24).

The complex of Fc and FcRIII was prepared by mixing both components in a 1:1 molar ratio and concentrating to 8–15 mg/ml for crystallization. Single crystals of orthorhombic and hexagonal forms were obtained by vapor diffusion in hanging drops at room temperature under slightly different crystallization conditions. Rod-shaped crystals of the orthorhombic form were grown from 10% polyethylene glycol 4000 and 50 mM Hepes at pH 6.0. Crystals were first obtained by vapor diffusion in hanging drops at room temperature and concentrating to 8–15 mg/ml for crystallization. Single crystals of orthorhombic and hexagonal forms were grown in two forms under different conditions. The orthorhombic crystal resulted in an unambiguous solution with a correlation coefficient (CC) of 39% and an R-factor (Rf) of 51% (CC = 51% and Rf = 49%) after rigid-body refinement in AmoRe (26). Molecular replacement searches using the hexagonal crystal data yielded a solution for the Fc from the third highest rotation solution that became the highest ranking translation solution with CC = 38% and Rf = 52% (CC = 46% and Rf = 50%) after rigid-body refinement in AmoRe (26). The position of FcRIII was determined in both crystal forms using the program EPMR (27) with a polyalanine model of FcRIII and the position of the Fc molecule fixed. Clear solutions were obtained for both crystal forms with CC = 56% and Rf = 48% for orthorhombic crystal and CC = 55% and Rf = 48% for hexagonal crystal, respectively. After rigid-body refinement of individual domains of the FcRIII-Fc complex modeled as polyalanine using CNS (28) most side chains had clear electron density into which side chains were built in. Disordered side chains lacking electron density were built with occupancies set to zero. The positional and grouped B-factor refinement was carried out using maximum likelihood as a target function with CNS version 0.9. Model adjustments and rebuilding were done using the program O (29). Carbohydrate molecules were added manually using 2Fo–F electron density maps contoured at 1.0σ and refined. The final model includes residues 5–172 of FcRIII, residues 235–444 for one chain of Fc, and residues 233–443 for the other chain of Fc.

**RESULTS AND DISCUSSION**

**Overall Structure of the Complex**—Crystals of a human FcRIII receptor in complex with a human Fc fragment of IgG1 were grown in two forms under different conditions. The orthorhombic crystals belong to the space group P2₁2₁2₁ and diffract to 3.0 Å resolution, whereas the hexagonal crystals have P6₃22 space group symmetry and diffract to 3.5 Å resolution. The structure of the complex was determined by molecular replacement in both forms and refined to their resolution limit. The final R-factors are R cryst = 28.0% and R free = 31.2% for the orthorhombic form and R cryst = 24.9% and R free = 32.6% for the hexagonal form, respectively (Table I). The electron density is continuous throughout the complex in the final 2Fo–F map except for three surface loops of FcRIII (residues 31–34, 99–105, and 142–149) located opposite from the Fc interface region. Despite different crystal packing and solvent

| Data collection | Orthorhombic form | Hexagonal form |
|-----------------|-------------------|---------------|
| Space group     | P2₁2₁2₁           | P6₃22         |
| Unit cell parameters (Å) | a = 73.8 | a = 114.9 |
| b = 102.4 | b = 102.8 |
| c = 123.1 | c = 123.3 |
| Resolution limit (Å) | 3.0 | 3.5 |
| Unique reflections | 19063 (1859) | 15541 (1511) |
| Redundancy | 4.8 (4.6) | 4.7 (4.8) |
| Completeness (%) | 99.9 (99.8) | 98.7 (99.4) |
| R cryst (%) | 61.0 (46.0) | 8.7 (42.3) |
| R free (%) | 75.0 (65.0) | 20.2 (4.0) |

**Data collection and refinement statistics**

| Refinement | Orthorhombic form | Hexagonal form |
|------------|-------------------|---------------|
| Resolution (Å) | 10–3.0 | 10–3.3 |
| No. reflections | 17780 | 11948 |
| No. nonhydrogen atoms | 4722 | 4733 |
| No. atoms in carbohydrate moieties | 202 | 185 |
| R cryst (%) | 23.0 (39.3) | 22.5 (31.3) |
| R free (%) | 28.9 (50.8) | 27.4 (36.5) |
| Mean B-factor (Å²) | 81 | 54 |
| Wilson B-factor (Å²) | 82 | 82 |
| r.m.s.d. bond lengths (Å) | 0.011 | 0.009 |
| r.m.s.d. bond angles (°) | 1.82 | 1.51 |

**Values for highest resolution shells, 3.00–3.11 and 3.30–3.44 Å for orthorhombic and 3.50–3.60 Å for hexagonal forms, are given in parentheses.**

**R cryst, R free** is calculated using test set of 5% of the reflections for the orthorhombic and 3% for hexagonal data sets.
D1 and D2 domains of Fc 

The Structure of FcγRIII—The structure of FcγRIII in both the orthorhombic and the hexagonal crystal forms can be readily superimposed with the structure of ligand free receptor resulting in r.m.s. differences between the individual domains of 0.6–0.8 Å among all Cz atoms (Fig. 2B) (22). The hinge angle between the N-terminal (D1) and the C-terminal (D2) domains is 60°, which is slightly larger than the 50° value observed in the ligand free receptor. However, no significant change in the receptor conformation is observed upon complex formation (Fig. 2B).

The Structure of Fc—The Fc fragment of an IgG1 antibody comprises two identical chains (A and B), and each consists of two C1-type immunoglobulin domains, C1α2 and C1α3. The overall shape of the Fc fragment resembles that of a horseshoe with the two C1α3 domains packing tightly against each other at the bottom of the horseshoe and the C1α2 domains held apart by carbohydrate moieties attached to the glycosylation site Asn297 from both chains forming the opening of the horseshoe. Well defined electron density throughout the Fc allowed for unambiguous tracing of residues Leu233 to Ser444 in chain A and Pro232 to Leu443 in chain B of Fc, including the lower hinge regions, Leu234–Pro238. The structure of the Fc fragment in complex with FcγRIII does not differ significantly from that observed in the structures of an unbound Fc fragment and a murine intact IgG2a antibody (30, 31) (Fig. 2C). However, the 2-fold symmetry relating the two chains of Fc in other unligated Fc structures, is no longer retained in the structure of the complex. The horseshoe-shaped Fc is slightly more open at the N-terminal end of the C1α2 domains in the FcγRIII-Fc structure compared with other known structures of Fc. The hinge angle between C1α2 and C1α3 domains of chain A (Fc-A) is 95° and 100° in the orthorhombic and the hexagonal crystals, respectively, ~10° larger than the corresponding angle of chain B (Fc-B) and the 84°–89° angle observed in all structures of ligand-free Fc (Fig. 2C).

The Interface between FcγRIII and Fc—The receptor binds to Fc at the center of the horseshoe opening making contacts to the lower hinge regions of both A and B chains of Fc (designated here as Hinge-A and Hinge-B, respectively, for the lower hinges of Fc-A and Fc-B) (Fig. 1). Such binding breaks down the dyad symmetry of the Fc, creating an asymmetric interface whereby the identical residues from Hinge-A and Hinge-B interact with different, unrelated surfaces of the receptor. Furthermore, it excludes the possibility of having a second receptor interacting with the same Fc molecule, resulting in a 1:1 stoichiometry for the receptor-Fc recognition. The structural implications of the activation of Fc receptors is profound. Particularly, the 1:1 receptor-Fc binding stoichiometry highlights the importance of antigen in the receptor aggregation. In contrast to the high affinity FcγRI and FcγRII receptors, the binding of immunoglobulins to FcγRIII in the absence of antigen does not lead to receptor aggregation. It can be argued that a 1:1 receptor-ligand stoichiometry ensures the need for antigens in forming the receptor aggregation by eliminating the possibility of Fc-mediated receptor aggregation as suggested in a 2:1 stoichiometry. Precluding receptor aggregation mediated by Fc alone also eliminates the potential deleterious effect of antibodies whose concentration in vivo are often much higher than that of antigen.

The receptor-Fc complex buries ~1453 Å² of solvent-accessible area (Fig. 3A). The interface between FcγRIII and Fc molecules shows poor shape complementarity with a mean shape correlation statistic of 0.53 (32), less than those between T-cell antigen receptor and Class I major histocompatibility complex molecules, between adhesion receptor CD2 and CD58, and between antibody and antigen complexes. On the receptor side, all the contacts to Fc are made exclusively through its D2 domain. The receptor D1 domain is positioned above the Fc-B and makes no contacts with Fc (Fig. 1A). The interface of the complex consists of the hinge loop between the D1 and D2 domain of the receptor, the BC, C′E, and FG loops, and the C′ β-strand. The BC loop is positioned across the horseshoe opening making contact with residues of both Hinge-A and Hinge-B. The C′-strand is situated atop the Fc-A leading to the C′E loop in contact with residues of Hinge-A. The FG loop of FcγRIII protrudes into the opening between the two chains of Fc (Fig. 1A). All three receptor loops (BC, C′E, and FG) were implicated in Fc binding through earlier studies of chimeric FcγRII/FcγRII receptors and through site-directed mutagenesis (9, 10, 12). On the Fc side of the complex, interactions with the receptor are dominated by residues Leu233–Pro238 of the lower hinge (Table II), consistent with results form earlier mutational studies (2). In particular, Hinge-A and -B together contribute ~60% of the overall receptor-Fc interface area (Fig. 3A). Interestingly, both Hinge-A and -B are found disordered in all known Fc structures to date, including the structure of an intact mouse IgG2a (30, 33–35). In contrast, residues of both Hinge-A and -B are clearly visible in the electron density maps from both crystal forms, suggesting that the binding of FcγRIII stabilizes the lower hinge conformation of Fc.

A combination of salt bridges, hydrogen bonds, and hydrophobic interactions contributes to the receptor-Fc recognition. Specifically, the interface between FcγRIII and Fc-A is dominated by hydrogen bonding interactions, whereas the hydrophobic interactions occur primarily at the interface between FcγRII and Fc-B. There are a total of nine hydrogen bonds between the receptor and Fc, forming an extensive network involving both the main-chain and side-chain hydrogen bonding interactions (Fig. 3, B and C, and Table II). Seven hydrogen bonds are distributed across the receptor and Fc-A interface and two are at the receptor and Fc-B interface. Alanine mutations, such as the H134A mutant of FcγRII that resulted in the loss of two interface hydrogen bonds, have been shown to re-
duce the receptor-Fc binding drastically, illustrating the importance of the interface hydrogen bonding network to the stability of the complex (10). A hydrophobic core is formed between Trp^90, Trp^113 of the receptor, and Pro^235 of the C12 domain of Fc-B (Fig. 3C). This hydrophobic core extends further to include Val^158, the aliphatic side chain of Lys^161 of the receptor and Leu^235 of Hinge-B. Mutations of both Trp^113 and Lys^161 in FcRIII lead to the loss in receptor function (11, 36). The side chain of Leu^235 on the Fc-B packs tightly against Gly^159 of the receptor leaving little space to accommodate any residues larger than Gly at this position. A G159A mutation on chimeric FcRIII resulted in the complete disruption of Fc binding, presumably due to the steric hindrance between Leu^235 and the β-carbon of the alanine mutant at position 159 (9). Of particular interest is Trp^113 of the receptor, which when mutated to Phe resulted in the loss of Fc binding. This residue is not only part of the interface hydrophobic core but also functions as a wedge inserted into the D1 domain to stabilize the acute interdomain hinge angle between D1 and D2 domains of FcRIII. A W113F mutation would result in the loss of this wedge and lead to a disruption in binding by altering the orientation between the D1 and D2 domains.

Comparison of the Structures of Receptor-Fc Complexes—Including the two crystal forms described in this work, there are a total of four Fc receptor and Fc complex structures available to date. A comparison among these structures reveals the conformation flexibility of this receptor-ligand complex and helps to explain the molecular interactions that differentiate the high from the low affinity receptors.

The two crystal forms of FcRIII-Fc complexes determined in the present study are essentially identical and can be readily superimposed with a root mean square (r.m.s.) deviation of 1.1 Å among all Cα atoms. The superposition of the hexagonal form onto the published FcRIII-Fc complex resulted in an r.m.s deviation of 0.5 Å for all Ca atoms (37) (Fig. 4A). An analysis of the interdomain hinge angles shows that the C12-C13 hinge angle is 10° larger in the structure of the FcRIII-Fc complex than it is in the structure of an intact IgG2a antibody (35) or the structures of ligand-free Fc (30) (Table III). This result is in a slightly more open conformation of the Fc when ligated to the receptor. Apart from the small change in the hinge angle, neither the Fc nor the receptor displays significant conformational change upon complex formation. The agreement between the orthorhombic and hexagonal crystal forms of the complex

**Fig. 2.** A, superposition of an α-carbon trace of the FcRIII-Fc complex determined from both the orthorhombic (green and cyan for FcRIII and Fc, respectively) and hexagonal (orange and red) crystal forms. B, superposition of the structure of FcRIII in the receptor-Fc complex (green) with that of ligand free receptor (orange). C, superposition of the structure of Fc in FcRIII-Fc complex (cyan) with that of unbound Fc (red).
The comparison between the structure of the FcγRIII-Fc complex and that of the FcεRI-Fc complex has provided further insight into the molecular basis of the receptor affinity (38). Overall, a similar mode of receptor-ligand recognition was observed in both the FcγRIII-Fc and the FcεRI-Fc complexes with an r.m.s. deviation of 1.5 Å between all the Ca atoms. In fact, most of the structural difference resulted from the small variation between the Cα2-Cα3 and Cε3-Cε4 interdomain hinge angles (Fig. 4, B and C). This angle is ~10° smaller in the FcεRI-Fc complex structure, resulting in a slightly closed conformation of Fc compared with that of the FcγRIII-Fc complex.

Detailed structural analysis shows that the interface area buried in the high affinity FcεRI-Fc complex (1850 Å²) is 400 Å² more than that in the low affinity FcγRIII-Fc complex (1453 Å²). This is primarily due to a more extensive interaction observed between the receptor and the non-lower hinge residues of Fc in the high affinity complex than in the low affinity receptor complex. Of the total interface area of the Fc, the lower hinge and non-lower hinge regions contribute 870 and 580 Å², respectively, in the FcγRIIIFc structure. The corresponding regions contribute 740 and 1110 Å², respectively, in the FcεRI-Fc structure. This results in approximately twice as much interface area contributed by non-lower hinge residues in the high affinity receptor-ligand complex than in the low affinity receptor-ligand complex. Structurally, the lower hinge of IgE-Fc adopts a very different conformation than that of IgG-Fc. Among the four IgG subtypes, the length of the hinge region is longest in IgG3 and shortest in IgG2 and IgG4 (three to four residues shorter than that of IgG1). The differences in both the lower hinge, possibly restricting the lower hinge conformation in IgE. Three other interface residues, Lys120, Tyr132, and Val158, are nearly invariant among all human Fc receptors. The limited variation observed can be easily modeled into the existing interface without creating steric hindrance. It is interesting that the interface salt bridge between Lys120 and Asp265 of the Fc appears to be absent in FcγRI but conserved in all other Fcγ receptors and in FcεRI. The other six interface residues, Hse83, Asp120, His134, His135, Arg155, and Lys161, are less well conserved among the receptors. Of these, variation at His134 and His135 may result in conformational changes in the lower hinge region of bound Fc. Overall, key features of the receptor-Fc interface appear to be well preserved among all the Fc receptors with possible hinge conformational adjustment for each receptor-Fc pair. Of particular interest is the comparison between the interface residues of FcεRI and those of FcγRIII. The binding affinity of FcγRIII is at least 100-fold weaker than that of FcεRI. Among the receptor-Fc interface residues, only four are different between FcγRIII and FcεRI. These are Lys120, Tyr132, Arg155, and Lys161 in FcγRIII and Asn120, Phe132, Ser155, and His161 in FcεRI. It is, however, not clear if any of these residues contribute to the observed variation in binding affinity.

The Fc Receptor IgG Subtype Specificities—Fcγ receptors display IgG subtype specificities. In particular, human FcγRIII binds tighter to IgG1 and IgG3 than it does to IgG2 and IgG4. Most of the Fc residues in contact with the receptor are conserved among the IgG subclasses (Fig. 5B, residues boxed in blue and red), suggesting a conserved binding site for all human IgGs. These binding residues, with the exception of a Gly269 to Asp replacement, are also conserved in murine IgG2a consistent with it being a ligand for human Fcγ receptors. The sequence differences among the IgG subclasses exist primarily at the lower hinge region. First, IgG2a has a Val and Ala at positions 234 and 235, respectively, instead of Leu and Leu as observed in IgG1 and IgG3, and a one-residue deletion at position 237 of the corresponding IgG1. Human IgG4 has a Phe at position 234 (Fig. 5B). In addition, IgG2 and IgG4 sequences contain a three-residue deletion relative to IgG1 at the N-terminal end of the lower hinge, possibly restricting the lower hinge conformation. The length of the lower hinge has been suggested as a factor in lower receptor binding affinity of IgG2 and IgG4 (2). Among the four IgG subtypes, the length of the hinge region is longest in IgG3 and shortest in IgG2 and IgG4 (three to four residues shorter than that of IgG1). The differences in both the

### Table II

| FcγRIII | Fc | Chain | Distance (Å) |
|--------|----|-------|--------------|
| Hydrogen bonds and salt bridges | | | |
| Thr116 O | Leu236 O | B | 3.3 |
| Lys120 N | Ser239 O | A | 3.4 |
| Lys120 N | Asp265 O | A | 3.2 |
| Lys120 N | Gly237 O | A | 3.4 |
| His134 N | Gly236 O | A | 3.5 |
| His134 N | Gly237 O | A | 2.4 |
| Hydrophobic contacts | | | |
| Ile98 O | Ala330 | B | |
| Trp90 O | Pro329 Gly236 | B | |
| Trp113 O | Pro329 | B | |
| His134 O | Gly236, Gly237, Asp265 | A | |
| Val118 O | Leu235 | B | |
| Gly229 O | Leu235 | B | |
| Lys219 O | Gly236 | B | |

---

*a* Carbon-carbon contacts ≤ 4.0 Å.
amino acid composition and the length of lower hinge may contribute to the observed lower receptor binding affinity of IgG2 and IgG4.

The Contribution of Carbohydrate to the Fcγ Receptor-Fc Binding—Both Fcγ receptors and antibodies are glycosylated in vivo. In contrast to the Fc fragment that displays only one conserved carbohydrate attachment site located at Asn\(^{297}\), the receptor glycosylation sites vary both in number and in location among different Fcγ receptors. For example, the glycosylation sites on the C-terminal domain of FcγRIII located at Asn138 and Asn145 on FcγRIII, Asn138 and Asn145 on FcγRII and asparagines 138, 145, and 149 on FcγRI (Fig. 5A). The influence of glycosylation on the receptor-Fc binding kinetics and on the receptor function has been studied extensively using both the deglycosylated receptor and Fc (4, 39). These studies demonstrated that the carbohydrate attached to Asn\(^{297}\) of Fc have a significant impact on the receptor binding, whereas glycosylations on the receptors appeared less critical and carbohydrates have more of a modulating effect on the affinity. For example, the two neutrophil antigen A alleles of FcγRIIIB, NA1 and NA2, differing primarily in their carbohydrate contents, display a 2-fold difference in their affinity for IgG3.

The structure of the receptor-Fc complex reveals potential roles for carbohydrate in receptor-Fc recognition. The first is the potential role of glycosylation at Asn\(^{297}\) in supporting the structural framework of the Fc. The Fc fragment used in this work was generated from a human IgG1 and is therefore glycanslated. Multiple carbohydrate moieties were visible in the electron density extending from Asn\(^{297}\) of both chains of Fc toward each other into the inter-chain region, referred to as the carbohydrate core region. Asn\(^{297}\) is located next to the receptor binding interface. The carbohydrate moieties, however, are orientated away from the interface making no specific contacts with the receptor. The glycosylation is thus unlikely to influence the receptor-Fc interface directly. However, the unique arrangement between the oligosaccharide moieties and the polypeptide chains of Fc makes it possible for the carbohydrate to affect the conformational stability of the receptor binding epitopes (40). Specifically, the spacing and the orientation between the two C\(_{1H2}\) domains may be influenced by the presence of sugar attachments (Fig. 1A). Because the binding of the receptor to Fc requires a particular orientation of the epitopes on both chains of Fc, it makes the receptor-Fc interface sensitive to the relative position and orientation of the two C\(_{1H2}\) domains.

A Model for FcγRIII-IgG Recognition—On a cell surface, the Fc receptor recognizes intact immunoglobulins. The presence of the Fab portion of antibody is likely to impose restrictions to the receptor-Fc recognition. To date, the only structure of an intact antibody available is that of a mouse IgG2a (31). Because FcγRIII also recognizes mouse IgG2a, a model of this receptor-antibody complex was generated by superimposing the Fc part of the current structure onto the Fc of the IgG2a (Fig. 6A). This receptor-antibody recognition model reveals that the receptor fits tightly and is nearly engulfed by the bound antibody.

Although the current structure offers an insight to antibody-Fcγ receptor recognition, the mechanism of receptor activation, namely the antigen-driven receptor clustering, remains unknown. Two receptor clustering models can be proposed based on the current structural results, a simple avidity model and an ordered receptor aggregation model (Fig. 6, B and C). The simple avidity receptor activation model assumes that the binding of oligomeric antigens by antibodies increases the
FIG. 5. A, sequence alignment of the membrane proximal domain of human Fc receptors. The secondary structure elements (arrow for β-strands and squiggle for α-helices) are indicated under the sequence. Residues identical to the sequence of FcγRIIIB are shown by periods, and gaps in sequence are shown by minus signs. Residues contacting the A and B chains of the IgG1 Fc are highlighted in red and blue boxes, respectively. The predicted N-linked glycosylation sites are indicated by asterisks. B, sequence alignment of the lower hinge and C\textsubscript{H}2 regions of human IgG1, 2, 3, and 4, a mouse IgG2a and a human IgE C\textsubscript{e}3 region. The residues contacting FcγRIII are highlighted in red and blue boxes for A and B chains of the Fc, respectively.

FIG. 6. Antibody-FcγRIII binding and ligand induced receptor aggregation model. A, an intact antibody-FcγRIII binding model. The structure of the antibody is shown in magenta and that of FcγRIII in green. The position of the second possible orientation of FcγRIII, which is in direct steric conflict with the hinge region and Fab, is indicated by a blue-shaded area. The arrow points to the location of the lower hinge (L.H.). The Protein Data Bank entry for the antibody coordinates is 1IGT. B, a simple avidity model of antigen-antibody binding induced FcγRIII aggregation. C, an ordered receptor aggregation model.
avidity as well as the proximity of the receptors, which is sufficient to its activation. The ordered receptor aggregation model assumes that the binding of oligomeric antibodies leads to the formation of an ordered receptor-ligand aggregation, which further stabilizes the activation complexes. Recent imaging studies on T cell and NK cell receptor activation processes suggest that the formation of the so-called immune synapse is an ordered event (41, 42). These results favor the structured aggregation model rather than the simple avidity model, although the molecular organization of Fcγ receptors during their activation remain to be determined. Recently, an ordered receptor-ligand aggregate was observed in the crystal lattice of a natural killer cell receptor in complex with its class I major histocompatibility complex ligand (43). Such a receptor-ligand aggregate is not observed in the two forms of the current FcγRIII-Fc crystals. However, a parallel receptor aggregate was observed in the crystal lattice of FcγRIII in the absence of Fc (22). A superposition of the current complex structure onto this lattice receptor aggregate suggests that the clustering model be compatible with the structure of a receptor-Fc complex (Fig. 6C).

Comparison of Fcγ Receptor with Other Ligands of Fc—The Fc region of the IgG molecule possesses multiple recognition sites for different components of immune system, including Fcγ receptors, neonatal Fc receptor (FcRn), rheumatoid factors (RF) and components of the complement system. In addition, it is also used as a ligand by staphylococcal proteins A and G. The structures of Fc complexed to FcRn, RF, protein A, and protein G are now known (30, 33, 34, 44). The binding of Fc by Fcγ receptors is characteristically different from all other known Fc ligands. First, the location of the Fc receptor binding site differs from those of neonatal Fc receptor, RF, and protein A. Although Fcγ receptors bind to the lower hinge region of Fc between the CH1 and CH2 domains, FcRn, RF, protein A, and protein G bind to the joint region between the CH2 and CH3 domains of Fc. Second, Fcγ receptors recognize Fc in an asymmetric fashion resulting in one receptor bound to both chains of Fc whereas all other ligands bind Fc in a symmetric fashion with each chain of Fc harboring an intact binding site (Fig. 7). The distinct binding site for Fcγ receptors suggests that it is possible to bind Fcγ receptors simultaneously with other ligands that recognize the CH2-CH3 joint region on the same Fc molecule. This raises the possibility of activation of multiple immune components by the same antigen-bound immune complex.

The recognition mode of binding to the lower hinge of Fc may evolve from the unique requirement of Fcγ receptor signaling, namely the need to have 1:1 recognition stoichiometry and to be capable of discriminating the IgG subtypes. Both CH2 and CH3 domains of Fc are very conserved among the subclasses of IgGs. Even the CH2-CH3 joint region, which is involved in binding of other Fc ligands, has near identical sequences among the IgG subclasses (Fig. 5B). The lower hinge region of IgGs, in comparison, is more variable allowing subtype-specific recognition of the receptor. The conformation of the hinge region, however, is quite flexible compared with the CH2 and CH3 domains of Fc (35). This hinge flexibility, which enables the Fab arms to adapt to the shape and form of antigens, may in fact hinder the binding of Fc receptors. Interestingly, there are two conserved cysteine residues forming two disulfide bonds at the N-terminal end of the lower hinge. The presence of these disulfides may stabilize the lower hinge conformation while allowing sufficient flexibility at the upper hinge region. Finally, the binding to the lower hinge region of both chains of Fc allows the receptor to monitor the integrity of the antibody.

Receptor-IgG Recognition and Autoimmune Diseases—In addition to their normal cellular functions in host immunity, FcγRs, in particular FcγRI and FcγRIII, also mediate the inflammatory responses generated by cytotoxic autoantibodies and immune complex triggered inflammatory disorders (45, 46). They provide a critical link to autoimmune diseases, such as rheumatoid arthritis, hemolytic anemia, and thrombocytopenia. The structure of FcγRIIB in complex with IgG1-Fc reveals the molecular interface of this receptor-Fcγ recognition and thus provides new possibilities for developing therapeutic reagents to block the activation of Fc receptors by autoantibodies. For example, the lower hinge sequence of Fc may be used to generate neutralizing antibodies that could block the binding of autoantibodies to FcγRs. The peptides encompassing residues of the BC and FG loops of the C-terminal domain of Fcγ receptors could also be used to develop neutralizing antibodies against the receptors. Finally, reagents that affect the glycosylation pathway may be used to affect the carbohydrate composition of Fc and thus the conformation of the receptor binding epitope.

Acknowledgments—We thank Dr. C. Hammer and M. Garfield for mass spectrometry measurements and N-terminal amino acid sequencing; Dr. J. Boyington and Dr. Z. Dauter for their assistance to the x-ray data collection at the National Synchrotron Light Source X2B beam line; and Dr. S. Ginell for his assistance at the Argonne National Laboratory Structural Biology Center 19-ID beamline at the Advanced Photon Source, whose use was supported by the U. S. Department of Energy, Office of Biological and Environmental Research, under Contract No. W-31-109-ENG-38.

REFERENCES

1. Daeron, M. (1997) Annu. Rev. Immunol. 15, 203–234
2. Tamm, A., and Schmidt, R. E. (1997) Int. Rev. Immunol. 16, 57–85
3. Holet, M. D., and Hogarth, P. M. (1994) Adv. Immunol. 57, 1–127
4. Galon, J., Robertson, M. W., Galinha, A., Mazieres, N., Spagnoli, R., Fridman, W. H., and Sautès, C. (1997) Eur. J. Immunol. 27, 1928–1932
5. Powell, M. S., Barton, P. A., Emmanouilidis, D., Wines, B. D., Neumann, H. M., Peiterzsch, G. A., Maxwell, K. F., Garrett, T. P., and Hogarth, P. M. (1999) Immunol. Lett. 68, 17–23
6. Kimberly, R. P., Ahlstrom, J. W., Click, M. E., and Edberg, J. C. (1990) J. Exp. Med. 171, 1239–1255
7. Unkeless, J. C., Shen, Z., Lin, C. W., and DeBeus, E. (1995) Semin. Immunol. 7, 37–44
8. Galon, J., Gauchat, J. F., Mazieres, N., Spagnoli, R., Storkus, W., Lotze, M.,
Bonnefoy, J. Y., Fridgen, W. H., and Sautes, C. (1996) J. Immunol. 157, 1184–1192
9. Hulett, M. D., Witort, E., Brinkworth, R. I., McKenzie, I. F., and Hogarth, P. M. (1994) J. Biol. Chem. 269, 15287–15293
10. Hulett, M. D., Witort, E., Brinkworth, R. I., McKenzie, I. F., and Hogarth, P. M. (1995) J. Biol. Chem. 270, 21188–21194
11. Tamm, A., Kister, A., Nolte, K. U., Gessner, J. E., and Schmidt, R. E. (1996) J. Biol. Chem. 271, 3659–3666
12. Cook, J. P., Henry, A. J., McDonnell, J. M., Owens, R. J., Sutton, B. J., and Klein, M. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9036–9040
13. Chappel, M. S., Isenman, D. E., Everett, M., Xu, Y. Y., Dorrington, K. J., and Jefferis, R. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 955–961
14. Kato, K., Matsunaga, C., Igarashi, T., Kim, H., Enokizono, J., Galinha, A., Kobayashi, Y., Fridman, W. H., Arata, Y., and Shimada, I. (2000) J. Mol. Biol. 295, 213–224
15. Lund, J., Winter, G., Jones, P. T., Pound, J. D., Tanaka, T., Walker, M. R., Artyukh, P. J., Arata, Y., Burton, D. R., and Jefferis, R. (1991) J. Immunol. 147, 2657–2662
16. Henry, A. J., Cook, J. P., McDonnell, J. M., Mackay, G. A., Shi, J., Sutton, B. J., and Koch, A., Esra, S., Radaev, S., Brooks, A. G., Fridman, W.-H., Sautes-Fridman, C., and Sun, P. D. (2000) Acta Crystallogr. Sect. D Biol. Crystallogr. 56, 265–278
17. Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998) Nature 395, 82–86
18. Davis, D. M., Chiu, I., Fasset, M., Cohen, G. B., Mandelboim, O., and Strominger, J. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 15062–15067
19. Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998) Nature 395, 82–86
20. Davis, D. M., Chiu, I., Fasset, M., Cohen, G. B., Mandelboim, O., and Strominger, J. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 15062–15067
21. Boyington, J. C., Motyka, S. A., Schuck, P., Brooks, A. G., and Sun, P. D. (2000) Nature 405, 537–543
22. Boros, P., Odin, J. A., Chen, J., and Unkeless, J. C. (1994) J. Immunol. 152, 379–383
23. Bonnafous, E., Fridgen, W. H., and Sautes, C. (1996) J. Immunol. 157, 1184–1192
24. Kato, K., Matsunaga, C., Odaka, A., Yamato, S., Takaha, W., Shimada, I., and Arata, Y. (1991) Biochemistry 30, 270–278
25. Tamm, A., Kister, A., Nolte, K. U., Gessner, J. E., and Schmidt, R. E. (1996) J. Biol. Chem. 271, 3659–3666
26. Navaza, J. (1994) Methods Enzymol. 276, 307–326
27. Kissinger, C. R., Gehlhaar, D. K., and Fogel, D. B. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 484–491
The Structure of a Human Type III Fcγ Receptor in Complex with Fc
Sergei Radaev, Shawn Motyka, Wolf-Herman Fridman, Catherine Sautes-Fridman and
Peter D. Sun

J. Biol. Chem. 2001, 276:16469-16477.
doi: 10.1074/jbc.M100350200 originally published online January 31, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100350200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 12 of which can be accessed free at
http://www.jbc.org/content/276/19/16469.full.html#ref-list-1