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Crystal structure of the soluble form of the human Fcγ-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 Å resolution

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Fcγ-receptors (FcγRs) represent the link between the humoral and cellular immune responses. Via the binding to FcγR-positive cells, immunocomplexes trigger several functions such as endocytosis, antibody-dependent cell-mediated cytotoxity (ADCC) and the release of mediators, making them a valuable target for the modulation of the immune system. We solved the crystal structure of the soluble human Fcγ-receptor IIb (sFcγRIIb) to 1.7 Å resolution. The structure reveals two typical immunoglobulin (Ig)-like domains enclosing an angle of ~70°, leading to a heart-shaped overall structure. In contrast to the observed flexible arrangement of the domains in other members of the Ig superfamily, the two domains are anchored by several hydrogen bonds. The structure reveals that the residues relevant for IgG binding, which were already partially characterized by mutagenesis studies, are located within the BC, C′E and FG loops between the β-strands of the second domain. Moreover, we discuss a model for the sFcγRIIb:IgG complex. In this model, two FcγR molecules bind one IgG molecule with their second domains, while the first domain points away from the complex and is therefore available for binding other cell surface molecules, by which potential immunosuppressing functions could be mediated.

Keywords: CD32/crystal structure/FcγRIIb/IgG receptor

Introduction

Fc receptors (FcRs) play a key role in defending the human organism against infections. After pathogens have gained access to the blood circulation they are opsonized by immunoglobulins (Igs). Due to their multivalency, the resulting immunocomplexes bind with high avidity to FcR-bearing cells leading to clustering of the FcRs, which triggers several effector functions (Metzger, 1992a). These include, depending on the expressed FcR type and associated proteins, endocytosis with subsequent neutralization of the pathogens and antigen presentation, antibody-dependent cell-mediated cytotoxity (ADCC), secretion of mediators or the regulation of antibody production (Fridman *et al.*, 1992; van de Winkel and Capel, 1993).

Specific FcRs exist for all Ig classes, with the ones for IgG being the most abundant and more diverse. Together with the high-affinity receptor for IgE (FcεRIa), FcγRI (CD64), FcγRII (CD32) and FcγRIIIa (CD16) occur as homologue type I transmembrane proteins or in soluble forms (sFcRs), but a glycosylphosphatidylinositol-anchored form of the FcγRIII (FcγRIIIb) also exists. Furthermore, FcγRs occur in various isoforms (FcγRIa, -b1, -b2, -c; FcγRIIa1-2, -b1-3, -c) and alleles (FcγRIIa1-HR, -LR; FcγRIIIb-NA1, -NA2) (van de Winkel and Capel, 1993). In contrast to the overall homologous extracellular parts, the membrane-spanning and the cytoplasmic domains differ. They may be deleted entirely or have a size of 8 kDa. They contain either a 26 amino acid immunoreceptor tyrosine-based activation motif (ITAM), as in FcγRIIa, or a respective 13 amino acid inhibitory motif (ITIM), in FcγRIIb, involved in signal transduction (Amigorena *et al.*, 1992).

Due to the conserved spacing of cysteines, the extracellular parts of the FcRs appear to consist of three (FcγRI, CD64) or two (FcεRI, FcγRII, CD32 and FcγRIII, CD16) Ig-like domains (10 kDa/domain) and therefore belong to the immunoglobulin superfamily. These highly glycosylated receptors are homologous and the overall identity in amino acid sequence among the FcγRs and FcεRIa exceeds 50% in their extracellular regions. Nevertheless, the affinity of FcR to their ligands varies widely. The higher affinity constant of ~10^8/M of the FcγRI to Fc fragment is assigned to its third domain, while the other FcγRs with two domains have an affinity constant to IgG varying between 10^5 and 10^7/M. The affinity constant of the two domain FcεRIa to IgE exceeds these values by far with 10^{10}/M (Metzger, 1992b).

FcγRs are expressed in a defined pattern on all immunologically active cells. FcγRI is constitutively expressed on monocytes and macrophages, and can be induced on neutrophils and eosinophils. The physiological role of FcγRI is still unknown as the expression on monocytes is not vital (Ceuppens *et al.*, 1988). The GPI-anchored form of the FcγRIII (FcγRIIIb) is exclusively expressed on granulocytes. Due to its missing cytoplasmic part, the signal transduction into the cell occurs solely via other transmembrane proteins such as complement receptor type 3 (CR3) that can at least associate with FcγRIIIb (Zhou *et al.*, 1993; Poo *et al.*, 1995). FcγRIIIa is expressed mainly on monocytes and macrophages, but only in conjunction with associated proteins (e.g. α- or γ-chains). FcγRII is the receptor with the widest distribution on immunocompetent cells and is mainly involved in the endocytosis of immunocomplexes.

FcγRIIa and FcγRIIb differ in their extracellular region by only 7% of the amino acid residues. Nevertheless, both forms can be distinguished by their binding characteristics to human and mouse IgG subclasses (van de Winkel and Capel, 1993) and their differing affinities to human IgGs (P.Sondermann, C.Kutscher, U.Jacob and J.Frey, in preparation). The situation becomes even more compli-

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cated by the high responder/low responder (HR/LR) polymorphism of FcγRIIa, named after the ability of T cells from some individuals to respond to murine IgG1-induced mitogenesis (Tax *et al.*, 1983). Later, it was found that the two exchanges in the amino acid sequence between the LR and the HR form modify the ability to bind human IgG2, which leads to the suggestion that at least one of them is involved in IgG binding (Hogarth *et al.*, 1992).

In contrast to the beneficial role FcRs play in the healthy individual, they also transmit the stimulation of the immune sytem in allergies (FcεRIa) or autoimmune diseases. Moreover, some viruses employ FcγRs to either gain access to the cells, e.g. HIV (Homsy *et al.*, 1989) and Dengue (Littaua *et al.*, 1990), or to slow down the immune response by blocking FcγRs, as in the case of ebola (Yang *et al.*, 1998) and measles (Ravanel *et al.*, 1997). Therefore, substances that prevent binding of immunocomplexes or viral particles to FcRs, thereby inhibiting cellular effector functions or retarding infections, are of clinical interest.

This interest has been substantially increased with the discovery of the soluble forms of the FcRs (sFcR) and their immunoregulatory potential over the last decade (Fridman *et al.*, 1993). These molecules are generated by proteolysis of membrane-bound FcγRs (Bazil and Strominger, 1994), by alternative splicing of the hnRNA (Rappaport *et al.*, 1993) or by separate genes (van de Winkel and Capel, 1993), and have been shown to suppress antibody production in cell culture (Varin *et al.*, 1989). Prolonged treatment of IgG-producing myeloma cells with sFcγRIII resulted in cytolysis (Hoover *et al.*, 1995). Because the concentration of sFcR in the above experiments was not sufficient to compete with the cell-bound form of the receptor for IgG, alternative ligands involved in signal transduction, as CR3 in the case of sFcγRIII (Lynch *et al.*, 1995), are discussed.

FcγRIIbs is active in the immune response, in contrast to the bacterial protein A (Deisenhofer *et al.*, 1978) or protein G (Sauer-Eriksson *et al.*, 1995), and the neonatal Fc-receptor (Burmeister *et al.*, 1994), which are not related to FcγRs and bind the Fc fragment via a helical domain. Therefore, the prototypic structure of the FcγRIIb presented here serves as a basis for the understanding of the molecular mechanisms in immunocomplex recognition of all members of the FcγR family and FcεRI.

Results

Structure determination

The crystal structure of recombinant soluble human FcγRIIb was solved by multiple isomorphous replacement (MIR) to 1.7 Å resolution, since a structure solution by molecular replacement with isolated domains of the Fc fragment from human IgG1 (Huber *et al.*, 1976; Deisenhofer, 1981, PDB entry 1fc1) failed. The putative extracellular part of the receptor (amino acid residues –4 to +180) was used for crystallization trials (P.Sondermann, R.Huber and U.Jacob, in preparation), while the model contains the residues 1–172 as the termini are flexible and not traceable into the electron density. Additionally, the model contains 150 water molecules and the refinement statistics are summarized in Table II. The structure contains a *cis* proline at position 11. None of the main-chain torsion angles are located in disallowed regions of the Ramachandran plot. The fully refined model was used to solve the structure of the same protein in crystals of space group $P4_22_12$ and of the glycosylated form derived from insect cells in crystals of space group $P3_121$ (Table I).

The polypeptide chain of FcγRIIb folds into two Ig-like domains as expected from a member of the immunoglobulin superfamily. Each domain consists of two β-sheets that are arranged in a sandwich, with the conserved disulfide bridge connecting the strands B and F on the opposing sheets (Figure 1). Three anti-parallel β-strands (A, B, E) oppose a sheet of five β-strands (C′, C, F, G, A′), whereby strand A leaves the three-stranded β-sheet and crosses over to the four-stranded anti-parallel sheet adding the short parallel fifth strand, A′. The arrangement of secondary structure elements as well as their connectivity is identical in both domains of the FcγRIIb and a rigid body fit of one domain onto the other revealed an r.m.s. distance of 1.29 Å of 67 matching C_α atoms.

The domains are arranged nearly perpendicularly to each other enclosing an angle of 70° between their long axes, forming a heart-shaped overall structure. This arrangement results in an extensive contact region between the domains (Figure 2). Residues from strand A′ and from the segment linking A′ and A of the N-terminal domain intermesh with residues of strands A1 and B from the C-terminal domain. This region is tightly packed and the interaction is strengthened by several hydrogen bonds resulting in a rigid arrangement which is conserved in the molecular structures seen in three different space groups. In orthorhombic, tetragonal and hexagonal (obtained with protein from insect cells) crystal forms, a deviation of $<2°$ in the interdomain angle is found.

Discussion

Overall structures

The structure of recombinant human FcγRIIb derived from *Escherichia coli* was solved by MIR to 1.7 Å resolution from orthorhombic crystals. An essentially identical structure is found in tetragonal crystals and with protein derived from insect cells in hexagonal crystals. In all three structures, the last nine residues of the polypeptide chain were found disordered. The flexibility of the C-terminal linker region between the structured core of the molecule and the transmembrane part may be functionally relevant in allowing some reorientation of the receptor to enhance the recognition of the Fc parts in immunocomplexes.

Homologue receptors

The Ig domains found in the Ig superfamily of proteins are characterized by a β-sandwich structure with a conserved disulfide bridge connecting two strands of the opposing sheets. The typical arrangement of three and four anti-parallel β-strands that form a sandwich, as found in FcγRIIb, occurs also in the T-cell receptor (Garcia *et al.*, 1996), Fc fragment (Huber *et al.*, 1976) and CD4 (Wang *et al.*, 1990). A structural alignment of the individual Ig domains of these molecules with the two domains of the FcγRIIb shows a common, closely related structure. However, the relative arrangement of the domains is not related in these molecules and covers a broad sector.

Table I. Data collection statistics

Derivative	Space group	No. of unique reflections	Multiplicity	Resolution (Å)	Completeness overall/last shell (%/%)	R_m (%)	No. of sites	Phasing power
NATI	$P2_12_12_1$	18 009	3.6	1.74	92.9/86.4	5.5		
NATI	$P4_22_12$	6615	4.5	2.70	97.1/94.3	10.1		
NATI-Baculo	$P3_121$	3545	2.5	3.0	93.0/98.9	14.4		
UOAc	$P2_12_12_1$	7722	4.2	2.1	96.8/95.7	7.3	1	1.79
PtPy	$P2_12_12_1$	5520	3.9	2.3	89.7/49.6	10.5	1	1.39

$R_m = \Sigma|I_h - \langle I_h \rangle|/\Sigma\langle I_h \rangle$
Phasing power: $\langle F_H \rangle/E$, where $\langle F_H \rangle = \Sigma(F_H{}^2/n)^{1/2}$ is the r.m.s. heavy-atom structure amplitude, where $E = \Sigma[(F_{PHC}-F_{PH})^2/n]^{1/2}$ is the residual lack of closure error with F_{PH} being the structure factor amplitude, and where $F_{PHC}=|F_P + F_H|$ is the calculated structure factor amplitude of the derivative.

Table II. Refinement statistics for data set NAT1 $P2_12_12_1$

Resolution range (Å)	8.0–1.74 Å
No. of unique reflections [F>0σ (F)]	16 252
R-factor	19.4
R_{free}[a]	27.9
No. of atoms per asymmetric unit	
protein	1371
solvent	150
R.m.s. deviation from ideal geometry	
bond length (Å)	0.009
bond angle (°)	2.007
Average B factors (Å^2)	
protein main chain	18.8
protein side chain	25.2
solvent	36.7
R.m.s. deviation of bonded B factors (Å^2)	4.1

[a]R_{free}: 5% of the reflections were used as a reference data set and were not included in the refinement.

Despite the structural similarity between Ig domains from different molecules and the strikingly low r.m.s. deviation of C_α atoms that result when the two domains of the FcγRII are superimposed, no significant sequence similarity is found (Figure 3A and B). A structure-based sequence alignment shows a conserved hydrophobicity pattern along the sequence of the domains together with, beside the cysteines, only few identical amino acid residues. We first prepared a structure based alignment of the two C-terminal domains of the IgG1 heavy chain and the FcγRIIb, and added the sequences of the other related FcγR and FcεRIa domains. This shows that the sequences of the three domain FcγRI and the two domain receptors are compatible with the hydrophobicity pattern of Ig domains, and several conserved amino acid residues are revealed. First, the different domains of a FcR are more related to each other than to Ig domains from other molecules of the Ig superfamily. Secondly, the N-terminal domains of the receptors relate to each other, as do the second domains. Thirdly, the sequence of the third domain of FcγRI shows features from both groups of domains. Taken together, we confirm the assignment of the FcRs to the Ig superfamily and speculate that all FcR domains originate from a common ancestor, an ancient single-domain receptor that acquired a second domain by gene duplication. Further divergent development of such a two-domain receptor resulted in the present diversity, including FcγRI which acquired a third domain.

Conservation of those amino acid residues that contribute to interdomain contacts in FcγRIIb in the alignment hint at a similar domain arrangement in different receptors. In Table III, the residues contributing with their side chains to the interdomain contact (Figure 2) are compiled for FcγRIIb, together with the corresponding amino acid residues in other receptors, according to the structure-based sequence alignment of Figure 3B. With the exception of Asn15, which is not conserved between the FcRs, the residues involved are identical or conservatively replaced providing strong support for a similar structure and domain arrangement in all FcRs. The third domain of FcγRI lacks these signature sequences and is probably arranged in a different manner.

The contact interface to IgG

Limited information on the interactions of FcRs with their ligands is available from mutagenesis studies (Hogarth *et al.*, 1992; Hulett *et al.*, 1994, 1995). By systematically exchanging loops between the β-strands of FcγRIIa for FcεRIa amino acid residues, the B/C, C′/E and F/G loops of the C-terminal domain were evaluated as important for ligand binding (Figures 1 and 3B). In the structure model, these loops are adjacent and freely accessible to the potential ligand. Additionally, most of the amino acid residues in these loops were exchanged for alanines by single-site mutations, which resulted in a drastic alteration of the affinity of FcγRIIa to dimeric human IgG1. Also, the single amino acid exchange Arg131 to His in the C-terminal domain (C′/E loop) in the high responder/low responder polymorphism, which alters the affinity of the FcγRIIa to murine IgG1, points to that region. Thus, the amino acid residues in this area are either important for ligand binding or the structural integrity of that region. Here, the structure shows a clustering of the hydrophobic amino acid residues Pro114, Leu115 and Val116 in the neighbourhood of Tyr157. This patch is separated from the region Leu159, Phe121 and Phe129 by the positively charged amino acid residues Arg131 and Lys117, which protrude from the core structure (Figure 3B).

Glycosylation

In the sequence of FcγRIIb three potential *N*-glycosylation sites are found. All three sites are on the surface of the molecule and are accessible. They are located in the E/F loops (N61 and N142) of both domains and on strand E (N135) of the C-terminal domain (Figures 1 and 4). Since

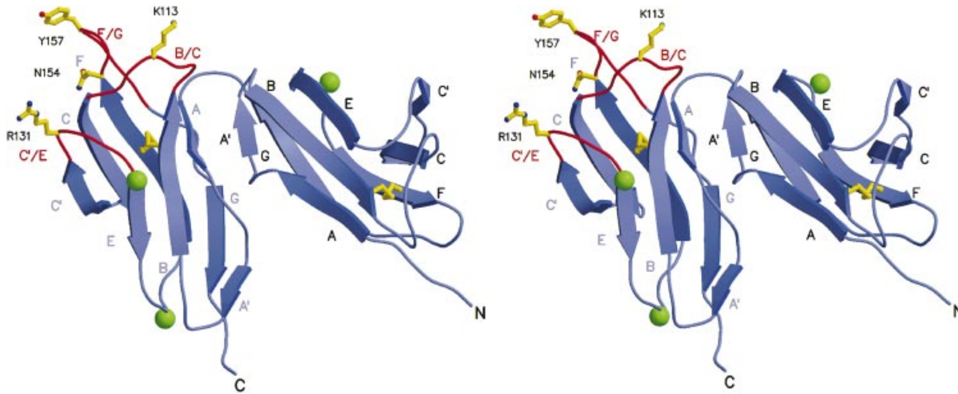


Fig. 1. Overall structure of human sFcγRIIb. Stereo ribbon representation of the sFcγRIIb structure. The loops supposed to be important for IgG binding are depicted in red with some of the residues within the binding site and the conserved disulfide bridge in ball and stick representation. The potential *N*-glycosylation sites are shown as green balls. The termini are labelled and the β-strands are numbered consecutively for the N-terminal domain in black and for the C-terminal domain in blue. The figure was created using the programs MOLSCRIPT (Kraulis, 1991) and RENDER (Merritt and Murphy, 1994).

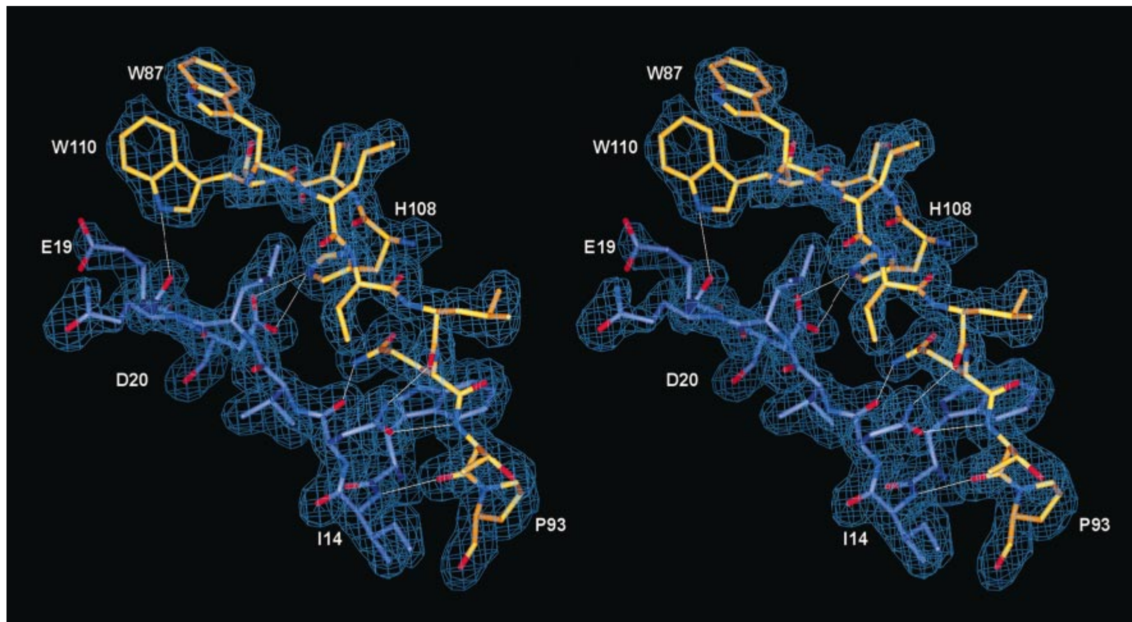


Fig. 2. Interdomain contacts. A close up of the residues involved in the interdomain contacts of sFcγRIIb. The amino acid residues of the N-terminal domain are depicted blue, and the residues of the C-terminal domain yellow. The model is covered by a $2F_o$–F_c electron density, contoured at 1 σ and obtained from the final coordinates. Hydrogen bridges between the domains are represented by white lines. The figure was created using the program MAIN (Turk, 1992).

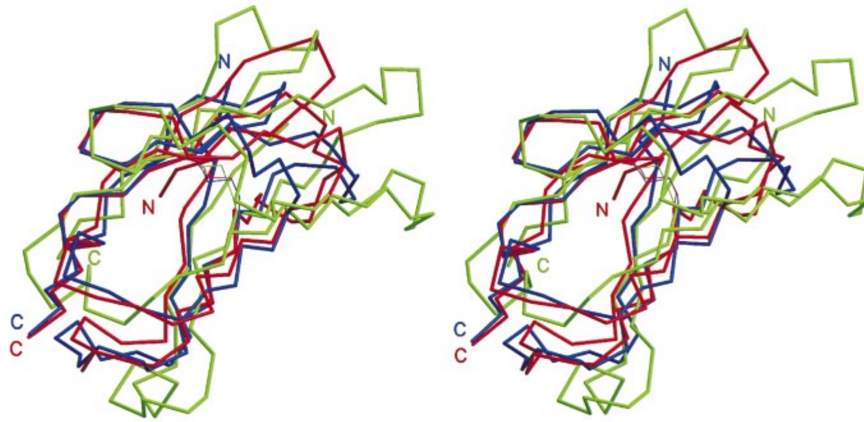
the material used for the solution of this structure was obtained from *Escherichia coli*, it does not contain carbohydrates, while the FcRs isolated from mammalian cells are highly glycosylated. The three potential glycosylation sites are located rather far from the putative IgG-binding region, and non-glycosylated FcγRIIb binds human IgG, suggesting a minor role of the glycosylation in binding. This was confirmed by the structure of the FcγRIIb produced in insect cells which is glycosylated (P.Sondermann, C.Kutscher, U.Jacob and J.Frey, in preparation). With the exception of a 2° change of the interdomain angle, possibly due to different crystal contacts, no differences between the glycosylated and unglycosylated protein structures were found. The three glycosylation sites are only optionally used, as shown by SDS–PAGE, where the material appears in four bands. No additional electron density for those sugars was found, presumably as a consequence of chemical and structural heterogeneity.

The IgG:FcγRIIb complex

The newly solved structure of FcγRIIb complements the information gained from the structure of the Fc fragment and the biochemical data available regarding the FcγR:IgG complex.

While diverse biochemical information concerning the binding site of the FcγRIIb (see above) is available, only limited data exist regarding the contact area contributed by the antibody. The IgG isotypes are closely related and exhibit graduated affinities to FcγRs. However, they still carry too many amino acid exchanges for the determination of the binding site, and mutational analyses led to some controversy. Single-site amino acid exchanges on IgG3 in the region on the CH2 domain directly following the hinge

A



B

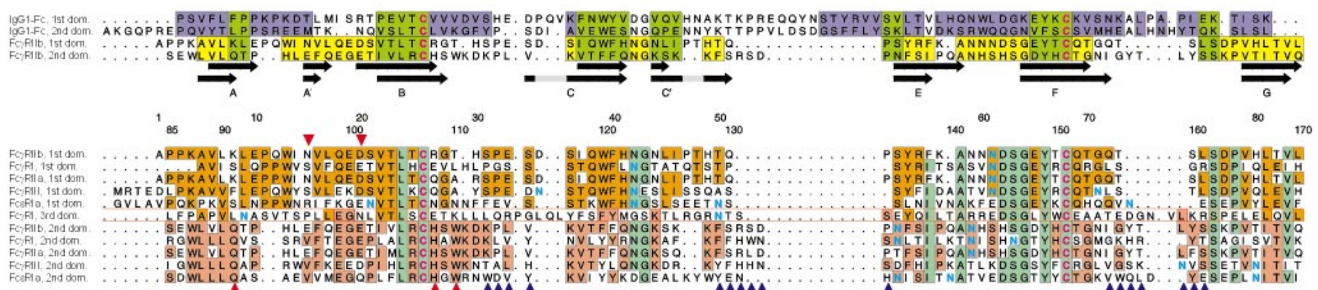


Fig. 3. (**A**) Superposition of the two FcγRIIb domains and the CH2 domain of human IgG1. Both domains of FcγRIIb and the CH2 domain of hIgG1 were superimposed. The N-terminal domain is depicted in blue, the C-terminal domain in red, and the CH2 domain of hIgG1 in green. The respective termini are labelled and the conserved disulfide bridges are depicted as thin lines. (**B**) Structure-based sequence alignment of the sFcγRIIb domains with domains of other members of the FcR family. The upper part of the figure shows the structure-based sequence alignment of the FcγRIIb and hIgG1 Fc-fragment domains performed with the program GBF-3D-FIT (Lessel and Schomburg, 1994). Amino acid residues with a C_α distance of <2.0 Å in the superimposed domains are masked: lilac, matching residues between the Fc-fragment domains; yellow, residues in the FcγRIIb domains; and green, when they can be superimposed in all four domains. The β-strands are indicated below this part of the alignment and are labelled as in Figure 1. The lower part of the figure shows the alignment of the amino acid sequences from the other FcγRs and the homologue FcεRIa to the profile given in the upper part of the figure using routines from the GCG package (Genetics Computer Group, 1994). The upper and lower row of numbering refer to the N- and C-terminal domains of FcγRIIb. The conserved cysteines are in magenta and the potential glycosylation sites in blue. Identical residues within the first domain are masked orange, those in the second domain pink and green when the residues are conserved within both domains. The less conserved third domain of FcγRI is aligned between the first and the second domains. Red arrows point to residues that are involved in side chain contacts between the first and the second domain while blue arrows depict residues that are relevant for IgG binding. The figure was produced with the program ALSCRIPT (Barton, 1993).

Table III. Residues that contribute to the interdomain contact via side chains

FcγRIIb	FcγRIIa	FcγRIII	FcγRI	FcεRIa
Asn15	Asn	Ser	Ser	Arg
Asp20	Asp	Asp	Glu	Glu
Gln91	Gln	Gln	Gln	Gln
His108	His	His	His	His
Trp110	Trp	Trp	Trp	Trp

region (lower hinge) of the antibody have resulted in altered affinities to FcγRI, FcγRII and FcγRIII (Jefferis *et al.*, 1990; Sarmay *et al.*, 1992). On the other hand, some authors favour the inter-domain cleft between the CH2 and CH3 domains of the antibody as a recognition site for the receptors. By exchanging homologue half domains between IgG1 and IgG4, which weakly binds FcγRIII, the C-terminal half of the CH2 domain was found to be sufficient for ADCC induction by FcγRIII (Greenwood *et al.*, 1993). In a further set of experiments, loops between the β-strands of the IgE domains were mutated to IgG1. It was shown that at least the A/B loop, which is located at the interface of the CE3 and CE4 domain (homologous to CH2 and CH3 in IgG), is important for FcεRI binding (Presta *et al.*, 1994; Helm *et al.*, 1996). Finally, the structure of protein A in complex with the Fcγ fragment shows that protein A binds to the connecting region between the CH2 and CH3 domains. Experiments with FcγR-bearing cells on which bound immuno-complexes could be displaced with protein A (Ades *et al.*, 1976) suggest an at least partially overlapping binding site of protein A and FcγRs on the antibody.

With the structures of both constituents available, we attempted to model the FcγRII:IgG complex using the program FTDock (Gabb *et al.*, 1997). FTDock uses Fourier correlation theory for evaluation of the shape and electrostatic complementarity of the complex component surfaces. In the hands of the authors, the programme has produced good results in predicting complex structures, but in some cases additional biochemical information on

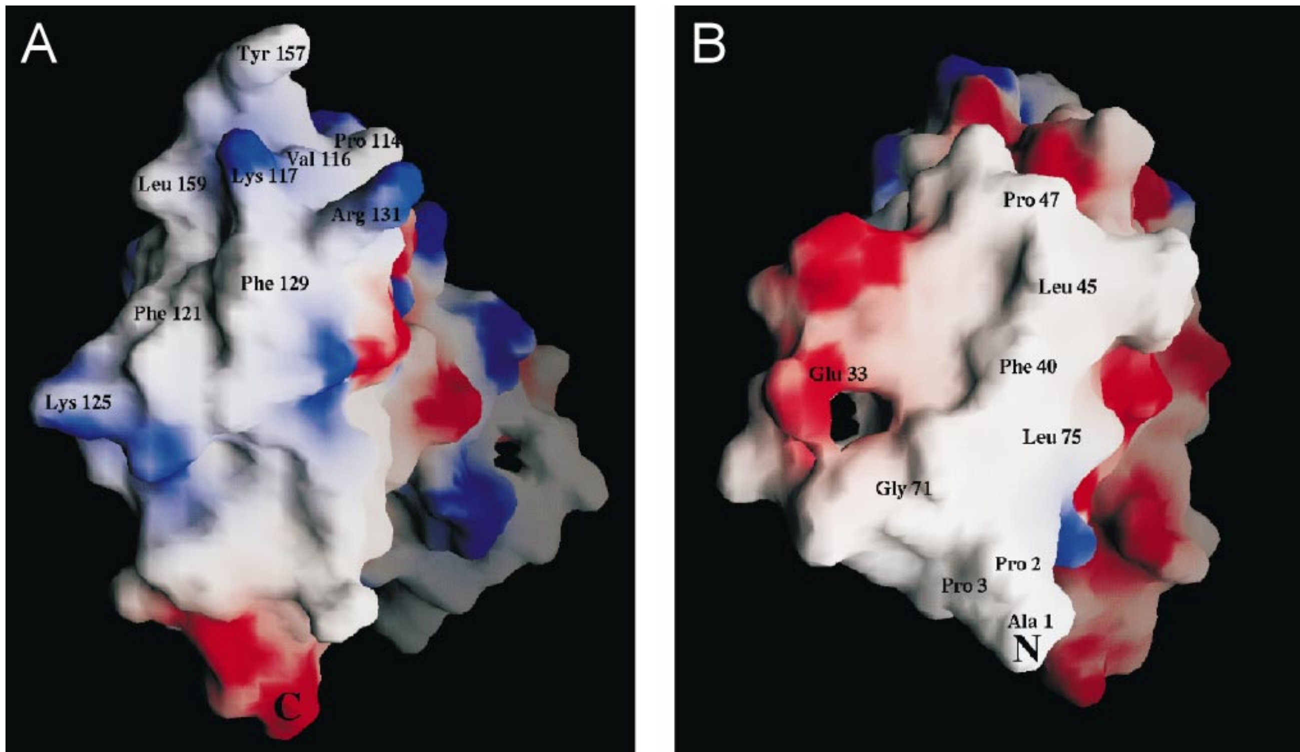


Fig. 4. The putative binding sites of FcγRIIb. Solid-surface representations of FcγRIIb as produced with GRASP (Nicholls *et al.*, 1991), with colour coding according to the relative surface potential from negative (red) to positive (blue). (**A**) The molecule as in Figure 1 by a rotation of ~90° counter clockwise around the vertical. (**B**) The molecule is rotated 90° clockwise around the same axis. Both views show the putative binding regions on the C-terminal (A) and the N-terminal domain (B). The amino acid residues discussed in the text are labelled.

the location of the contact area was needed to exclude false positive solutions. Without applying additional restrictions concerning the region of the contact surface between FcγRIIb and the Fc fragment our calculations resulted in a single solution clearly scoring above the rather constant background. The program predicted a complex structure with the B/C, C′/E and F/G loops of the FcγRIIb domain 2 contributing to the contact site as predicted by the mutagenesis experiments. The program suggests that FcγRIIb binds into the cleft between the CH2 and the CH3 domain of the IgG.

Taken together, we would suggest the following complex structure: two FcγRIIbs bind into the cleft between the CH2 and the CH3 domain of the antibody, employing the 2-fold symmetry of the Fc-fragment. The 2:1 stoichiometry between FcγRIIb and Fc-fragment in the complex could be shown in equilibrium gel filtration experiments (P.Sondermann, C.Kutscher, U.Jacob and H.Frey, in preparation). Such a complex could be positioned upright on the membrane, thereby leaving maximal conformational freedom to the Fab arms to be engaged in antigen binding. In contrast, an arrangement that would employ the lower hinge as binding region would enforce, due to a fixed distance between the membrane and the binding site on the FcγRII molecule, a flat-lying complex with restricted motional freedom of the Fab arms. The N-terminal domains of the receptors in our model point away from the complex and lie between the Fab arms when the complex is viewed along the Fc fragment towards the membrane (Figure 5). The surface in the N-terminal domain corresponding to the binding region of the C-terminal domain is accessible in the proposed complex and forms a large uncharged patch with a hydrophobic ridge comprising the amino acid residues Pro47, Leu45, Phe40, Leu75, Pro3, Pro2 and Ala1 (Figure 4B). This region might represent a binding site for other protein components that have been discussed for FcγRIIb to explain the signalling capabilities of its soluble form.

Thus, the proposed complex structure is consistent with the biochemical data available although mutational analysis of the antibody component favours a binding region close to the hinge. Mutations in that region which might have an indirect (allosteric) effect should be judged critically since alterations near the dimerization area between the heavy chains could lead to a disturbance in the arrangement of the CH2- and the CH3-domain. However, the unequivocal arrangement of the complex components and questions regarding the graded affinities of the FcR variants to the IgG isotypes, and the knowledge-based design of inhibitors that interfere with the recognition of immunocomplexes have to await the solution of the complex structure from crystals.

Materials and methods

Protein chemistry

Recombinant soluble human FcγRIIb was expressed in *E.coli*, refolded, purified and crystallized, as described elsewhere (P.Sondermann, R.Huber and U.Jacob, in preparation). Briefly, the putative extracellular region of hFcγRIIb2 (Engelhardt *et al.*, 1990) was overexpressed in *E.coli*. Inclusion bodies were purified by lysozyme treatment of the cells and subsequent sonification. The resulting suspension was centrifuged (30 min 30 000 *g*) and washed with buffer containing 0.5% *N*,*N*-dimethylamine-*N*-oxide (LDAO). A centrifugation step and resuspension in LDAO-

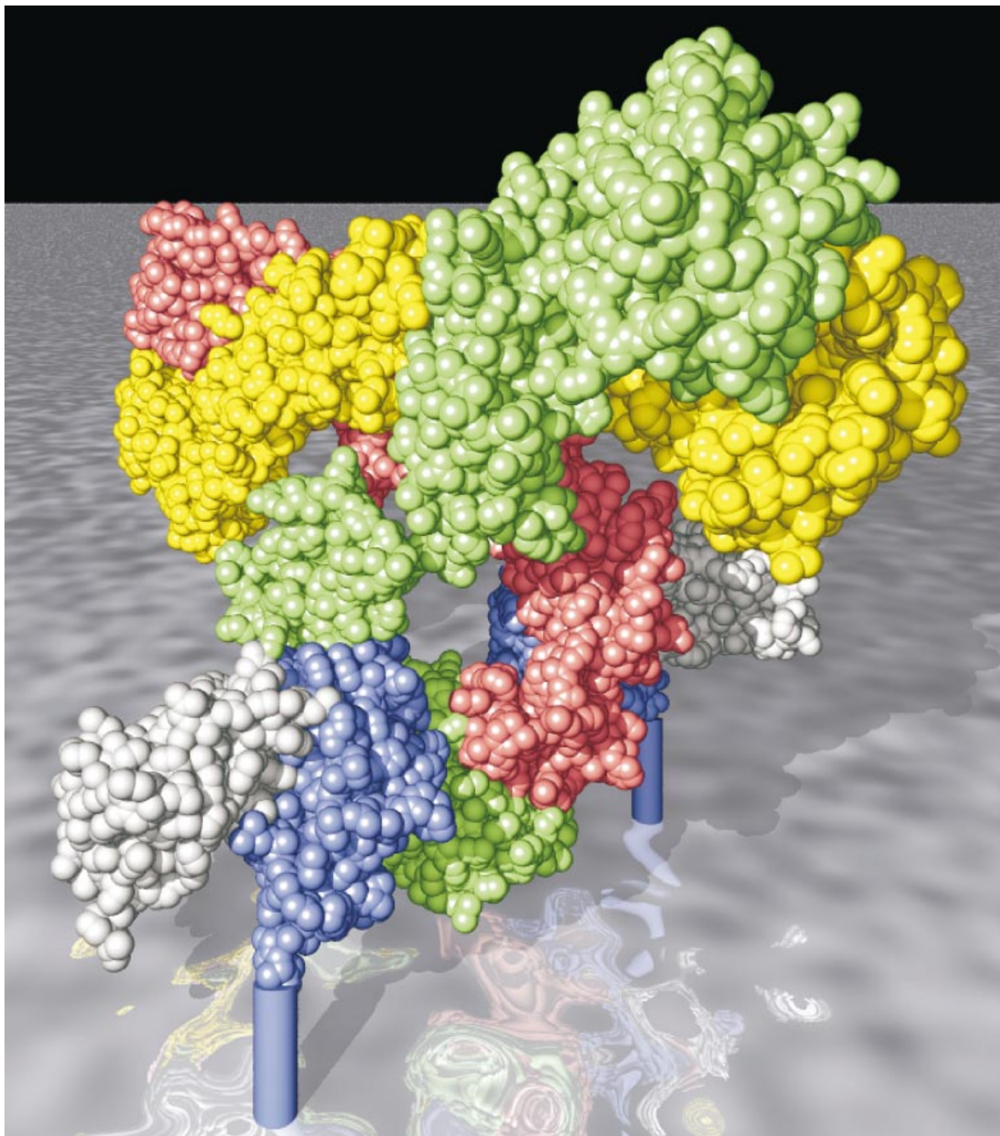


Fig. 5. Cartoon of the proposed FcγR–IgG complex The cartoon shows a complete complex of two FcγRIIb binding one antibody. The heavy chains of the antibody are depicted in red and green, and the light chains in yellow. The blue atoms represent the C-terminal domain of sFcγRIIb, while the white ones represent the N-terminal domain. A blue column connects the receptor to the membrane instead of the flexible nine amino acid linker region, which remained invisible in the electron density. The image was produced with the program POVRAY.

containing buffer was repeated once before this procedure was repeated twice without LDAO. The inclusion bodies were dissolved in 6 M guanidine hydrochloride and the protein renatured. The dialysed and filtered protein solution was applied to a hIgG–Sepharose column and eluted by pH jump. The concentrated neutralized fractions were subjected to size-exclusion chromatography on a Superdex-75 column (26/60; Pharmacia).

Crystallization

Crystallization was performed in sitting drops at 20°C using the vapour diffusion technique. Therefore, 3 μl of the protein solution of FcγRIIb (7 mg/ml), buffered in 2 mM [*N*-morpholino]propanesulfonic acid (MPS), 150 mM NaCl, 0.02% sodium azide, were added to 1.5 μl of the reservoir solution. Crystals grew to their final size within 3 to 7 days. The crystals used for data collection were grown in 33% PEG2000, 0.2 M sodium acetate pH 5.4 (orthorhombic form) and 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 10% (v/v) 1,4-dioxane, 100 mM sodium chloride (tetragonal form). The insect cell-derived protein was crystallized in 32% PEG6000, 0.2 M sodium acetate, pH 5.3.

Preparation of heavy-atom derivatives

The heavy-atom derivatives were prepared by soaking the crystals in the crystallization buffer containing 2 mM platinum(II)-(2,2′-6,2″terpyridinium)chloride for 24 h or 10 mM uranylacetate for 8 days.

X-ray data collection

Diffraction data was collected on an image plate system (MAR research) using graphite monochromated CuK_{α} radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM 5.50 (Leslie, 1997), and the data was subsequently scaled and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

Structure determination

The structure was solved with the standard procedures of the MIR method. From the large number of soaks carried out with different heavy-atom components only two compounds yielded interpretable Patterson maps. The heavy-atom positions for each derivative were determined from difference Patterson maps and initial phases were calculated. Cross-phased difference Fourier maps were used to confirm heavy-atom positions and to establish a common origin for the derivatives. Anomalous data were included to discriminate between the enantiomers. The heavy-atom parameters were further refined with the program MLPHARE from the CCP4 package, leading to the statistics compiled in Table I. An electron density map was calculated to a resolution of 2.1 Å, and the phases were improved further by solvent flattening and histogram matching with the program DM from the CCP4 suite. The

resulting electron density map was of sufficient quality to build most of the amino acid residues. Model building was performed with O (Jones *et al.*, 1991) on an Indigo2 work station (Silicon Graphics, Inc.). The structure refinement was done with XPLOR (Brünger *et al.*, 1987) by gradually increasing the resolution to 1.7 Å using the parameter set of Engh and Huber (1991). When the structure was complete, after several rounds of model building and individual restraint B-factor refinement (R-factor = 29% ; R_{free} = 36%), 150 water molecules were built into the electron density when a F_o–F_c map, contoured at 3.5 σ, coincided with well defined electron density of a $2F_o$–F_c map contoured at 1 σ. The resulting refinement statistic is shown in Table II. The coordinates have been deposited with the Protein Data Bank, Brookhaven, NY, under accession No. 2fcb and released 1 year after publication.

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