

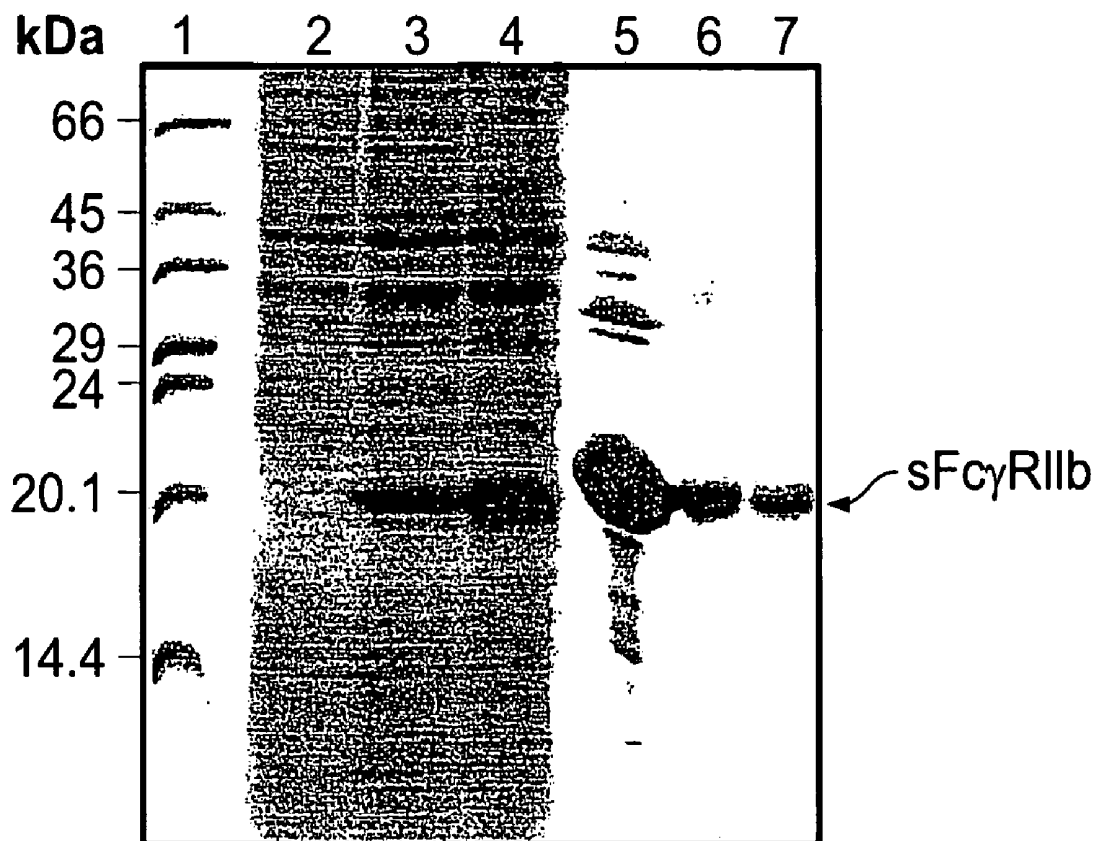


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(19) **United States**(12) **Patent Application Publication**
Sondermann et al.(10) **Pub. No.: US 2007/0207163 A1**(43) **Pub. Date: Sep. 6, 2007**(54) **RECOMBINANT SOLUBLE FC RECEPTORS****Publication Classification**(76) Inventors: **Peter Sondermann**, Krailling (DE);
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530/413; 536/23.1(21) Appl. No.: **11/327,695**(57) **ABSTRACT**(22) Filed: **Jan. 6, 2006****Related U.S. Application Data**(62) Division of application No. 09/856,933, filed on Feb.
27, 2002, now Pat. No. 7,074,896, filed as 371 of
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Dec. 3, 1998 (EP) 98 122 969.3

Recombinant soluble Fc receptors according to the present invention are characterized by the absence of transmembrane domains, signal peptides and glycosylation. Such Fc receptors can easily be obtained by expressing respective nucleic acids in prokaryotic host cells and renaturation of the obtained inclusion bodies, which procedure leads to a very homogenous and pure product. The products can be used for diagnostic as well as pharmaceutical applications and also for the generation of crystal structure data. Such crystal structure data can be used for the modelling of artificial molecules. A further embodiment comprises coupling the Fc receptors according to the invention to solid materials like chromatography materials that can be used to separate and/or enrich antibodies.



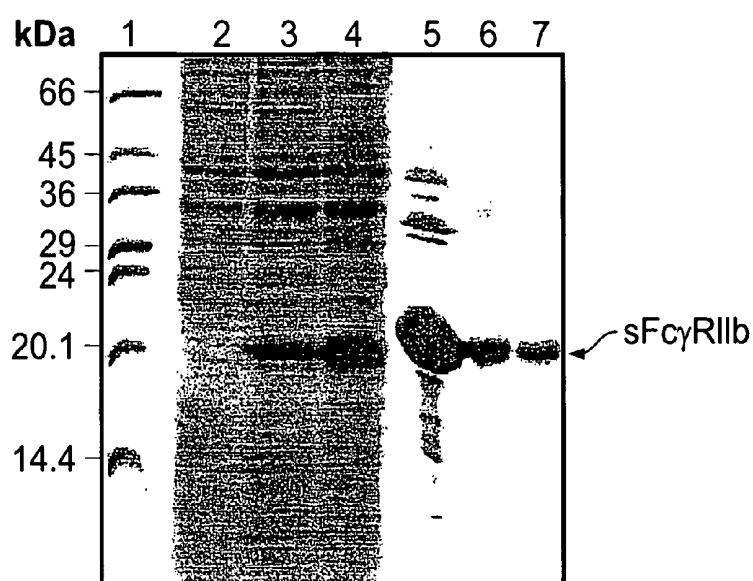


FIG. 1

Gel Filtration Experiment with PLS in Running Buffer (RB)

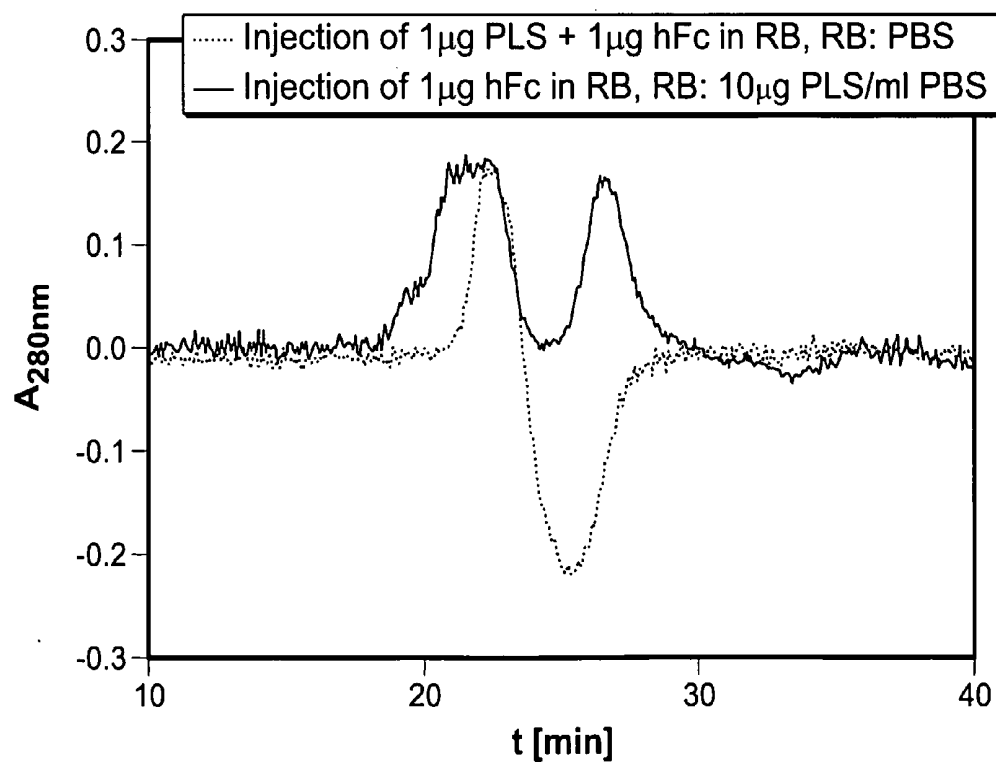


FIG. 2

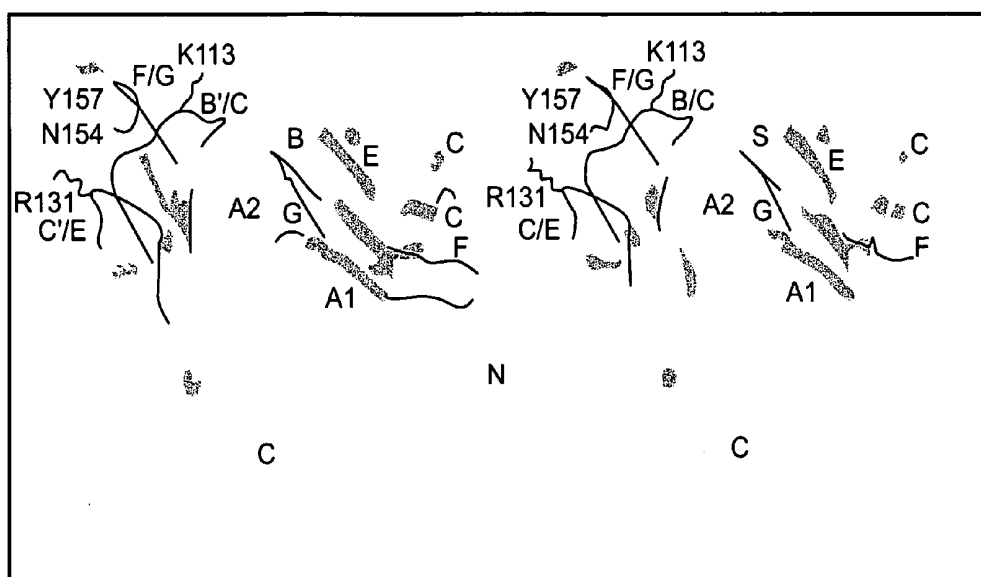


FIG. 3

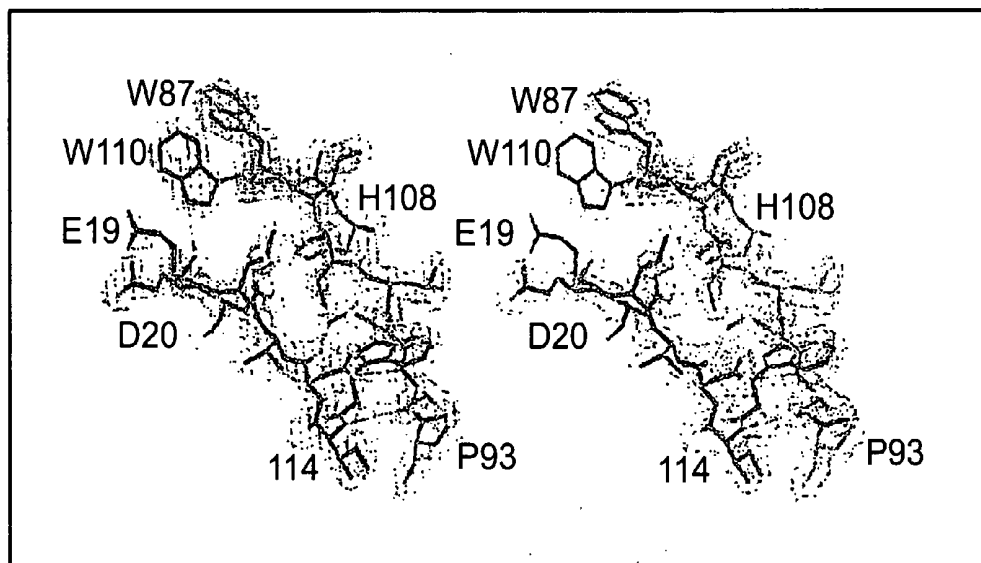


FIG. 4

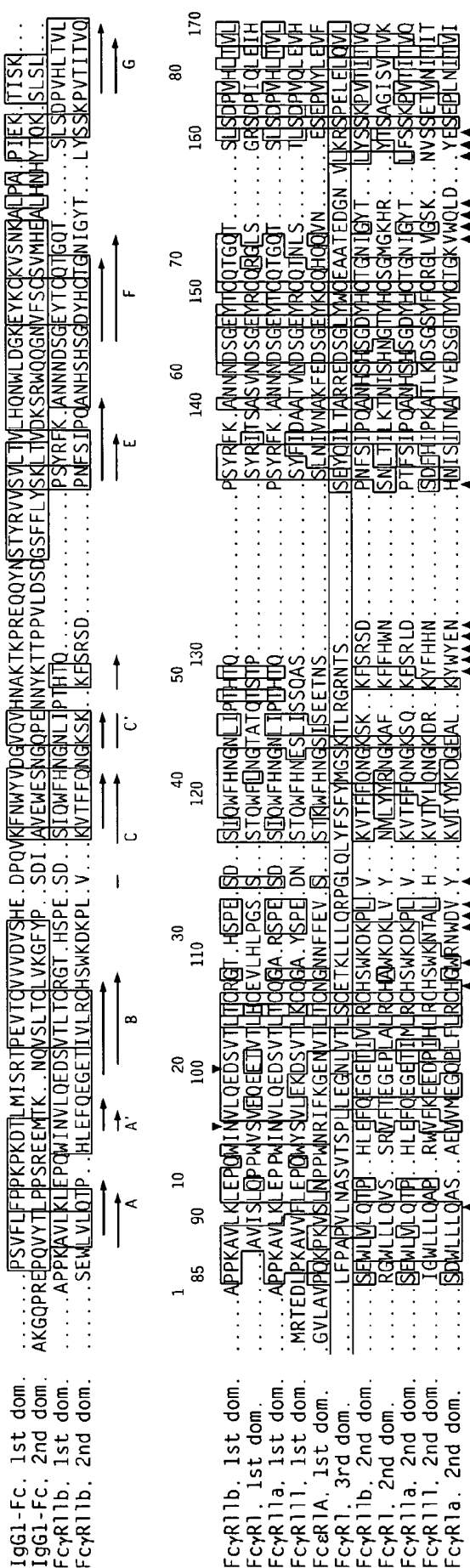


FIG. 5B

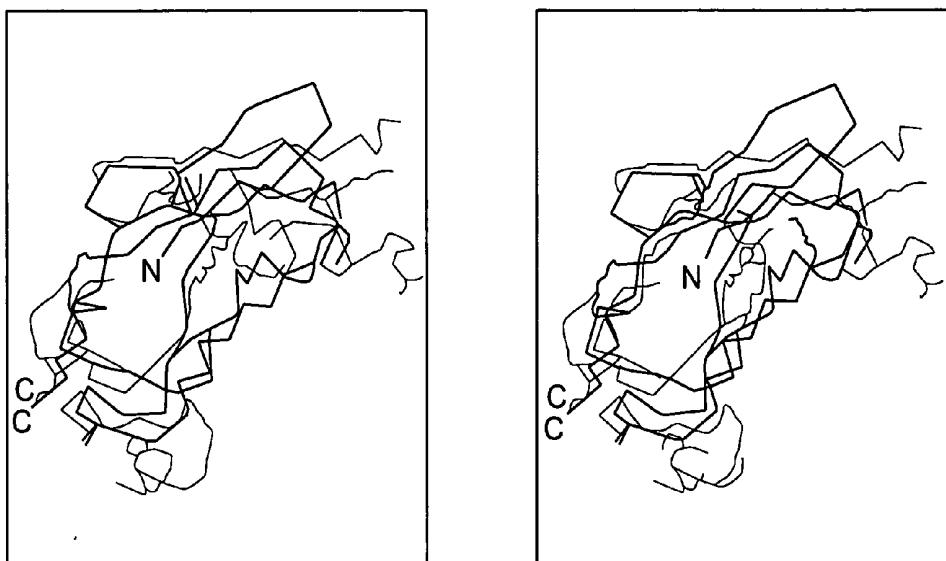


FIG. 5A

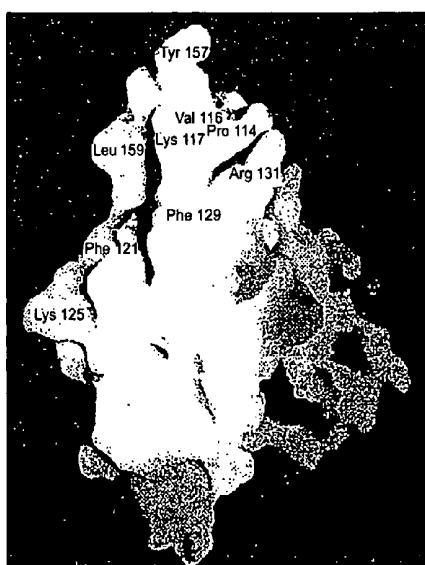


FIG. 6A

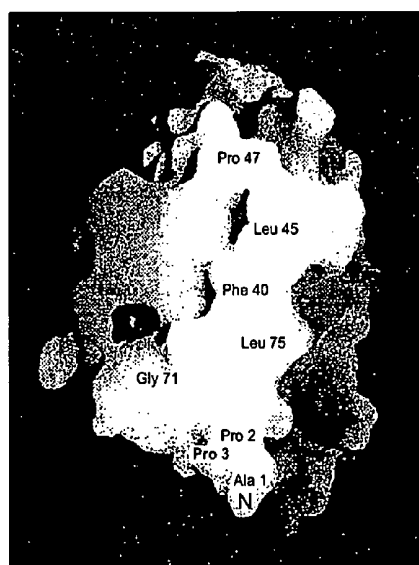


FIG. 6B

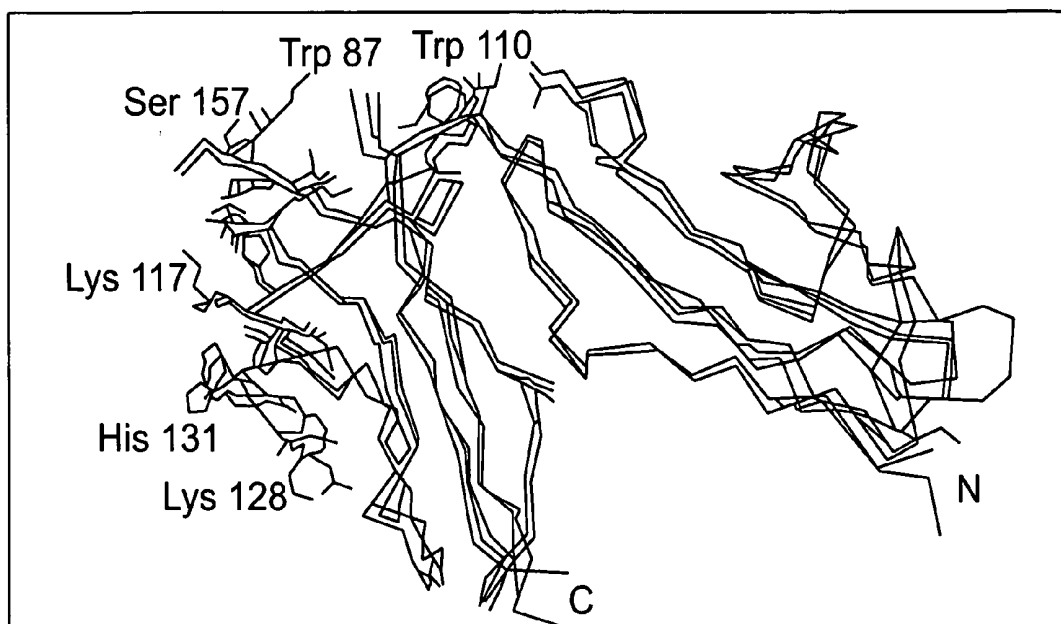


FIG. 7

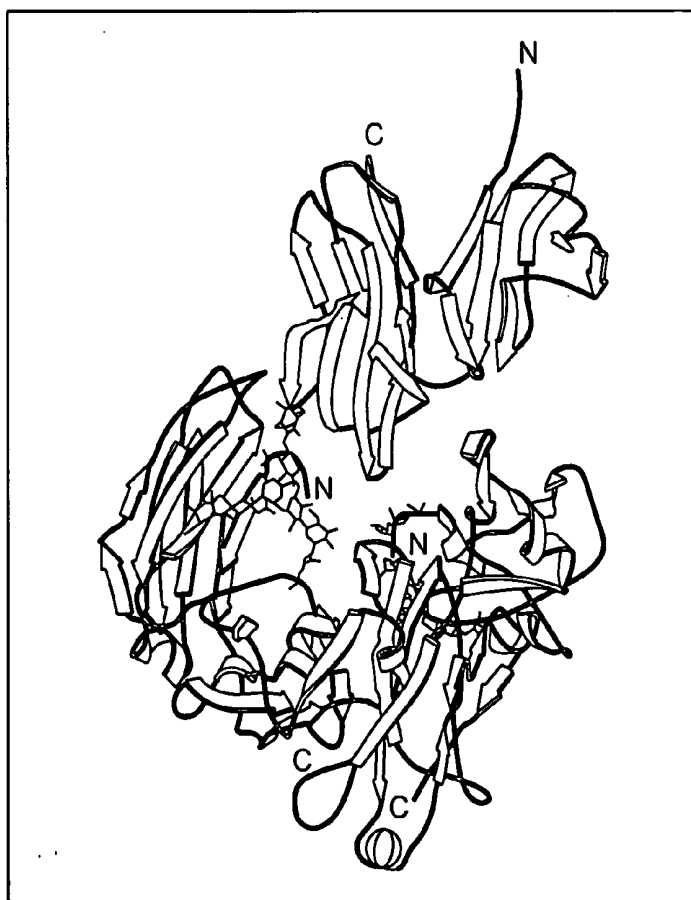


FIG. 8

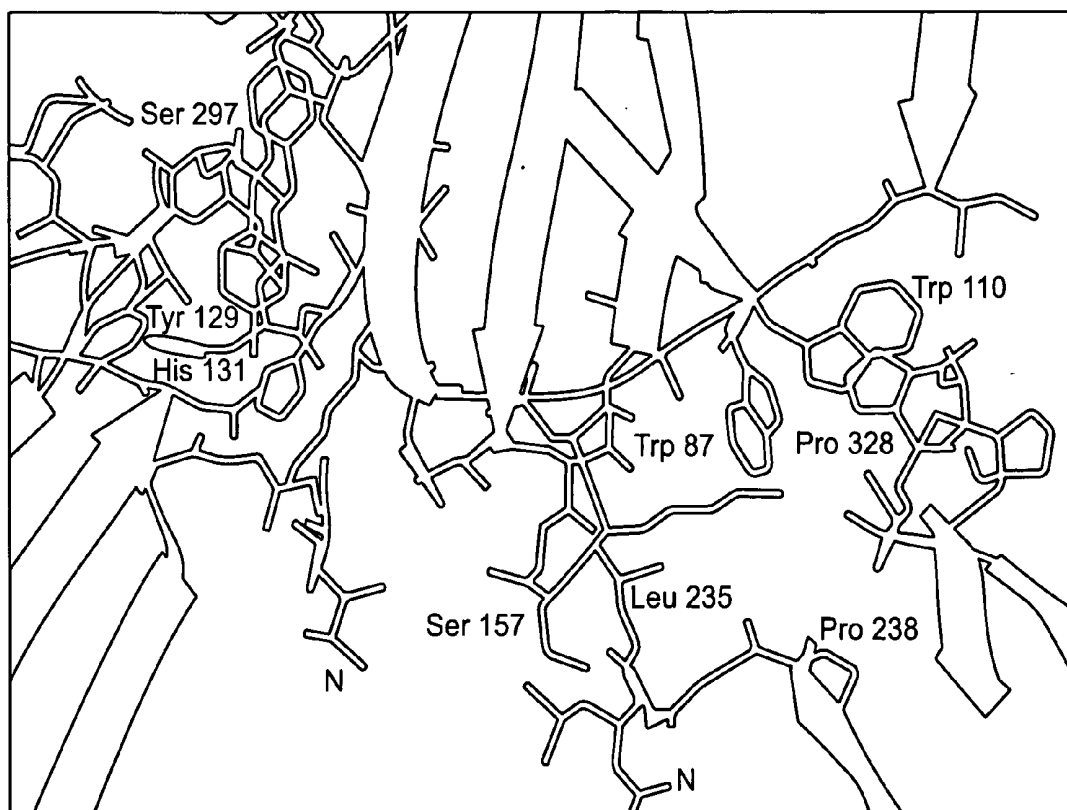
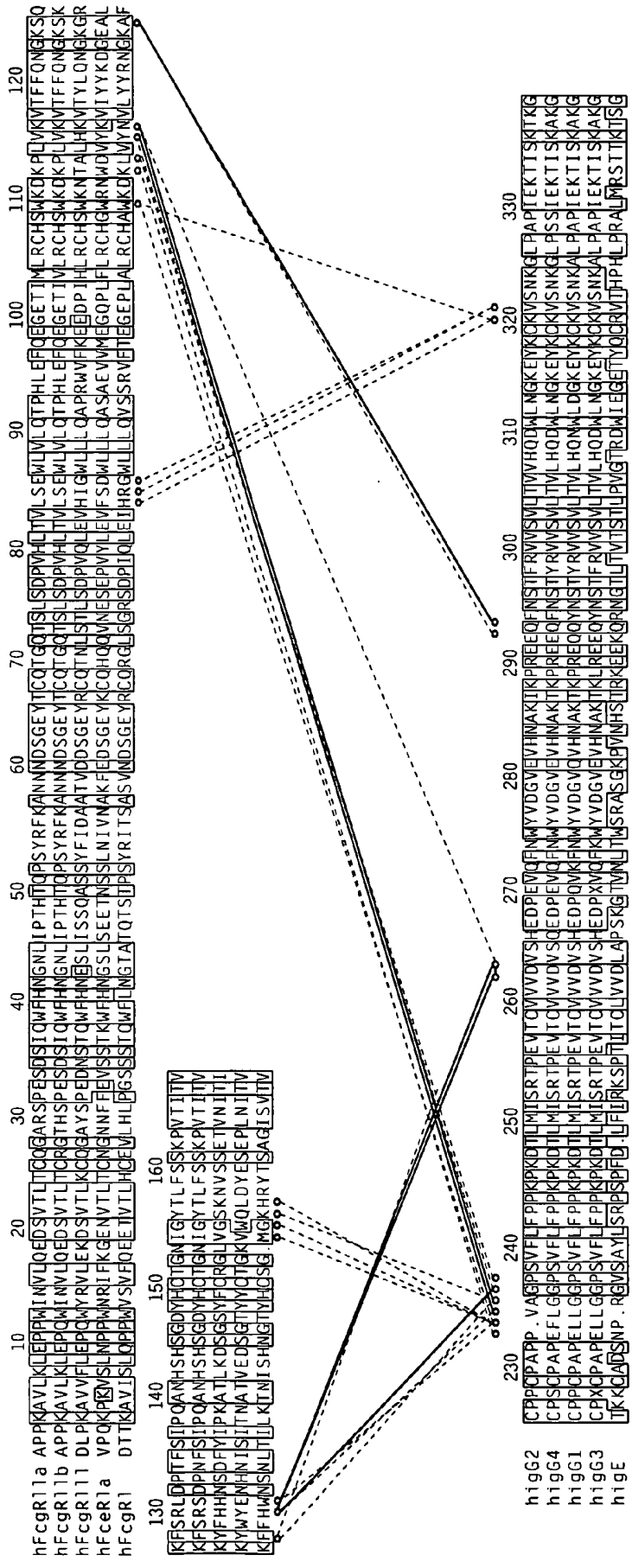


FIG. 9



FLG. 10A

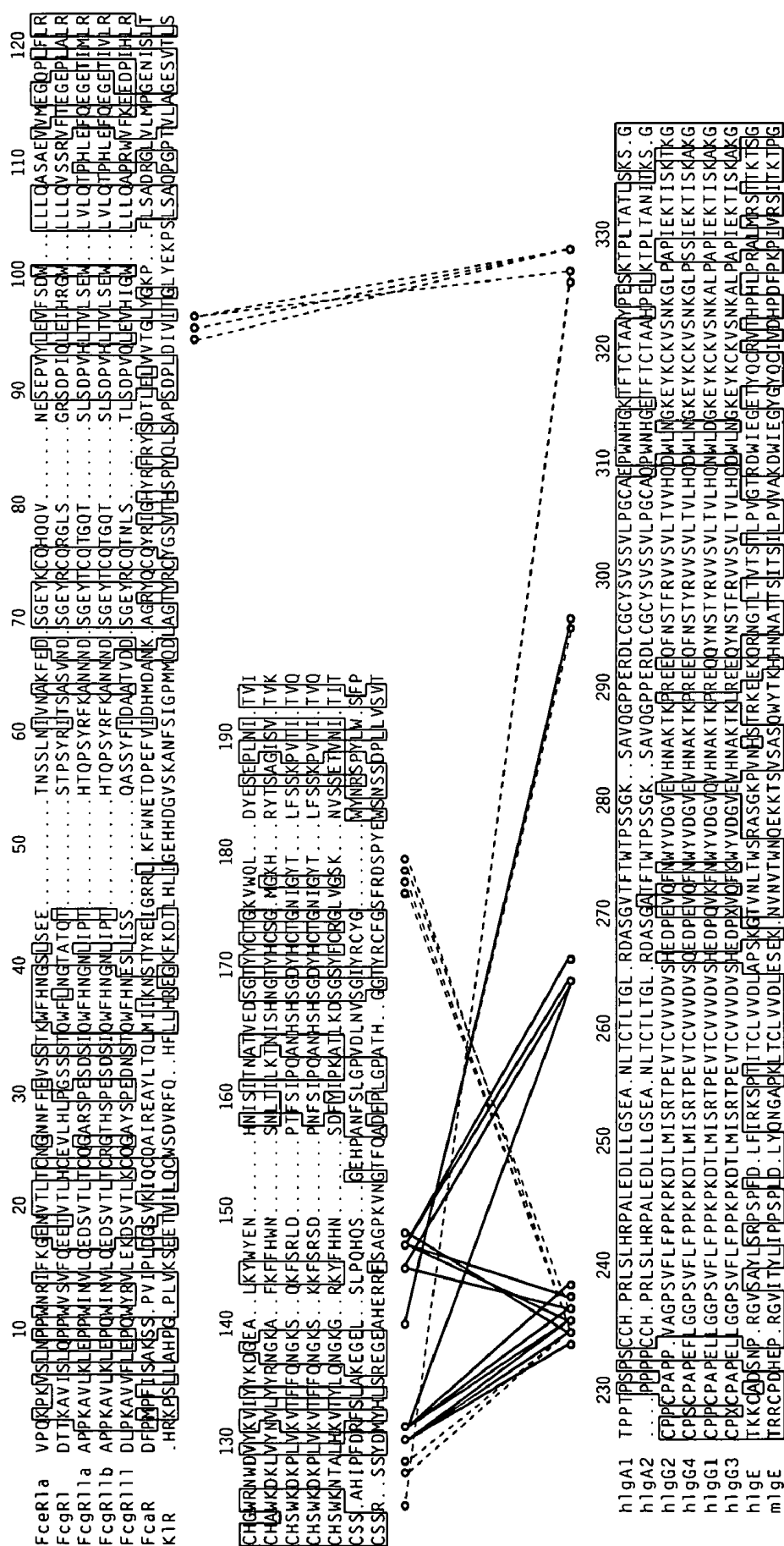


FIG. 10B

Alignment of the Produced sFcγR, sFcεR1a and the short form of sFcεR1I

sFcγRIIa	---MAAPPKAVLKLEPP-WINVLEQEDSVTLTCQGARSPESDSIQWFHN-GNLIPHTHTQPS	55
sFcγRIIb	MGTPAAPPKAVLKLEPQ-WINVLEQEDSVTLTCRGTHSPESDSIQWFHN-GNLIPHTHTQPS	58
sFcγRIII	-MRTEDLPKAVVFLEPQ-WYSVLEKDSVTLKCQGAYPEDNSTQWFHN-ESLISSQASSY	57
sFcγRI	-----MAVISLQPP-WVSVFQEETVTLHCEVLHLPGSSSTQWFLN-GTATQTSTPSY	50
sFcεR1a	---MAVPQKPKVSLNPP-WNRIFKGENVTLCNGNFFEVSSSTKWFFHN-GSLSEETNSSL	55
sFcεRII	-MELQVSSGFVNCNTCPEKWINFQRK-----C---YYFGKGTQKQVWHARYACDDMEGQLV	50
	* * . . : * . . : *	
sFcγRIIa	YRFKANNNDGSG-EYTCQTGQTSLSDPVHLTVLSEWLV-LQTPHLEFQEGETIMLRCHSWK	113
sFcγRIIb	YRFKANNNDGSG-EYTCQTGQTSLSDPVHLTVLSEWLV-LQTPHLEFQEGETIVLRCHSWK	116
sFcγRIII	FIDAATVNDGSG-EYRCQTNLSTLSDPVQLVHIGWLL-LQAPRWVFKEEDPIHLRCHSWK	115
sFcγRI	RITSASVNDGSG-EYRCQRLSGRSDPIQLEIHRGWLL-LQVSSRVFTEGEPLALRCHAWK	108
sFcεR1a	NIVNAKFEDSG-EYKQHQVQVNESEPVYLEVFSDWLL-LQASAEVVMGQPLFLRCHGWR	113
sFcεRII	SIHSPEEQDFTLKHASHTGSWIGLRNLDLKGEFIWVDGSHVDYSNWPAGEPTS-RSQGED	109
	. : * . . . : * * : . . * . .	
sFcγRIIa	DKPLVKVTFQNGK-SQKFSLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQ	172
sFcγRIIb	DKPLVKVTFQNGK-SKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQ	175
sFcγRIII	NTALHKVTYLQNGK-DRKYFHNSDFHIPKATLKDSGSYFCRGLVGSKNVSSETVNIITIT	174
sFcγRI	DKLVYNVLYYRNGK-AFKFFHWSNLITLKTNISHNGTYHCSG-MGKHRYTSAGISVTVK	166
sFcεR1a	NWDVYKVIYYKDGE-ALKYWYENHNISITNATVEDSGTYCTGKVVQLDYESEPLNITVI	172
sFcεRII	CVMMRGSGRWDAFCDRKLGAWCDRLATCTPPASEGSAESMGPDSPDPDGLPTPSAP	169
	: . . * : . * . * . . :	
sFcγRIIa	VP-----	174
sFcγRIIb	APSSSPMGII-----	185
sFcγRIII	QG-----	176
sFcγRI	ELFPAPVLNASVTSPLLEGNLVTLSCETKLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQ	226
sFcεR1a	KAPREKYWLQF-----	183
sFcεRII	LHS-----	172
sFcγRIIa	-----	
sFcγRIIb	-----	
sFcγRIII	-----	
sFcγRI	ILTARREDSGLYWCEAATEDGNVLKRSPLELQVLGLQLPTPV	269
sFcεR1a	-----	
sFcεRII	-----	

FIG. 11

Alignment the Produced sFcγR and sFcεR1a without sFcεR11

sFcγRIIa	---MAAPPKAVLKLEPPWINVLQEDSVTLTCQGARSPESDSIQWFHNGNLIPHTHTQPSYR	57
sFcγRIIb	MGTPAAPPKAVLKLEPQWINVLQEDSVTLTCRGTHSPESDSIQWFHNGNLIPHTHTQPSYR	60
sFcγRIII	-MRTEDLPKAVVFLEPQWYSVLEKDSVTLKCQGAYSPEDNSTQWFHNESSLISSQASSYFI	59
sFcγRI	-----MAVISLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYRI	52
sFcεR1a	---MAVPQKPKVSLNPPWNRIFKGENVTLCNGNNFFEVSTKWFHNGSLSEETNSSLNI	57
	. : * : * : * : : : : . * : * : * : .	
sFcγRIIa	FKANNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLTQPHLEFQEGETIMLRCHSWKDKPL	117
sFcγRIIb	FKANNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLTQPHLEFQEGETIVLRCHSWKDKPL	120
sFcγRIII	DAATVNDSGEYRCQTNLSTLSDPVQLEVHIGWLLQAPRWVFKEEDPIHLRCHSWKNTAL	119
sFcγRI	TSASVNDSGEYRCQRLSGRSDPIQLEIHRGWLLQVSSRVFTEGEPLALRCHAWKDKLV	112
sFcεR1a	VNAKFEDSGEYKCQHQVNESEPVYLEVFSDWLLQASAEVVMEGQPLFLRCHGWRNWDV	117
	* . : * * * * * * * : * : * : * : * : * : * : * : * : * : * : * : *	
sFcγRIIa	VKVTFQNGKSQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQVP---	174
sFcγRIIb	VKVTFQNGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQAPSSS	180
sFcγRIII	HKVTYLQNGKDRKYFHNSDFHPIKATLKDSGSYFCRGLVGSKNVSSETVNITITQG---	176
sFcγRI	YNVLYYRNGKAFKFFHWSNLTILKTNISHNGTYHCSG-MGKHRYTSAGISVTVKELFPA	171
sFcεR1a	YKVIYYKDGEALKYWYENHNISITNATVEDSGTYCTGKVVQLDYESEPLNITVIKAPRE	177
	: * : : * : * : : : * : : . . . * * * * * * : : * :	
sFcγRIIa	-----	
sFcγRIIb	PMGII-----	185
sFcγRIII	-----	
sFcγRI	PVLNASVTSPLEGNLVTLSCETKLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTAR	231
sFcεR1a	KYWLQF-----	183
sFcγRIIa	-----	
sFcγRIIb	-----	
sFcγRIII	-----	
sFcγRI	REDSGLYWCEAATEDGNVLKRSPLELQVLGLQLPTPV	269
sFcεR1a	-----	

FIG. 12

RECOMBINANT SOLUBLE FC RECEPTORS

[0001] The present invention relates to recombinant soluble Fc receptors (FcR), recombinant nucleic acids coding for such Fc receptors, host cells containing corresponding nucleic acids as well as a process for the determination of the amount of antibodies of a certain type contained in the blood, plasma or serum of a patient, a process for the determination of the immune status of patients with chronic diseases of the immune system and a process for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors. Further, the present invention is concerned with pharmaceutical compositions containing the recombinant soluble FcRs, crystalline preparations of FcRs and FcR/Ig-complexes and especially of the use of such crystalline preparation for the generation of crystal structure data of Fc receptors as well as FcR inhibitors and pharmaceutical compositions containing such FcR inhibitors.

[0002] A still further subject of the present invention is a recombinant Fc receptor coupled to a solid phase, e.g. a chromatography carrier material. The use of such chromatography material, which is another subject of the present invention, lies in the absorption of immunoglobulins from a body fluid of patients or from culture supernatants of immunoglobulin producing cells.

[0003] 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). The resulting immunocomplexes bind due to their multivalency with high avidity to FcR bearing cells leading to clustering of the FcRs, which triggers several effector functions (Metzger, H., 1992A). These include, depending on the expressed FcR type and associated proteins, endocytosis with subsequent neutralization of the pathogens and antigen presentation, antibody-dependent cellular cytotoxicity (ADCC), secretion of mediators or the regulation of antibody production (Fridman et al., 1992; van de Winkel and Capel, 1993).

[0004] Specific FcRs exist for all Ig classes, the ones for IgG being the most abundant with the widest diversity. Together with the high affinity receptor for IgE (FcεRIa), FcγRI (CD64), FcγRII (CD32) and FcγRIIIa (CD16) occur as type I transmembrane proteins or in soluble forms (sFcRs) but also a glycosylphosphatidylinositol anchored form of the FcγRIII (FcγRIIIb) exists. Furthermore, FcγRs occur in various isoforms (FcγRIa, b1, b2, c; FcγRIIIa1-2, b1-3, c) and alleles (FcγRIIIa1-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 be of a size of 8 kDa. They may contain either a 26 amino acid immunoreceptor tyrosine-based activation motif (ITAM) as in FcγRIIIa or a respective 13 amino acid inhibitory motif (ITIM) in FcγRIIIb involved in signal transduction (Amigorena et al., 1992).

[0005] Judged by the conserved spacing of cysteins, the extracellular part of the FcγRs consists of three (FcγRI, CD64) or two (FcεRI, FcγRII, CD32 and FcγRIII, CD16) Ig-like domains (10 kDa/domain) and therefore belongs to the immunoglobulin super family. These highly glycosylated receptors are homologues, and the overall identity in

amino acid sequence among the FcγRs and FcεRIa exceeds 50% in their extracellular regions. Nevertheless, the affinity of FcRs to their ligands varies widely. The higher affinity of $\approx 10^8 \text{M}^{-1}$ of the FcγRI to Fc-fragment is assigned to its third domain, while the other FcγRs with two domains have an affinity to IgG varying between 10^5 and 10^7M^{-1} . The affinity of the two domain FcεRIa to IgE exceeds these values by far with a constant of 10^{10}M^{-1} (Metzger, H., 1992B). In contrast to the mentioned FcRs the low affinity receptor for IgE FcεRII represents a type transmembrane protein and shows a lower homology.

[0006] FcγRs are expressed in a defined pattern on all immunological 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 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 like complement receptor type 3 (CR3) that can at least associate with FcγRIIIb (Zhou et al., 1993; Poo et al., 1995). FcγRIIIa is mainly expressed 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.

[0007] FcγRIIIa and FcγRIIIb 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 affinity to human IgGs (Sondermann et al., 1998A). The situation is rendered even more complicated by the high responder/low responder (HR/LR) polymorphism of FcγRIIIa 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).

[0008] In contrast to the beneficial role FcRs play in the healthy individual, they also transmit the stimulation of the immune system in allergies (FcεRIa) or autoimmune diseases. Moreover, some viruses employ FcγRs to get access to cells like HIV (Homsy et al., 1989) and Dengue (Littau et al., 1990) or slow down the immune response by blocking FcγRs as in the case of Ebola (Yang et al., 1998) and Measles (Ravanel et al., 1997).

[0009] Hence, the object underlying the present invention was to provide receptors which are easy to produce and can advantageously be used for medical or diagnostic applications. Moreover, it was an object of the invention to provide soluble receptors exhibiting a binding specificity and activity which is analogous to that of the receptors occurring naturally in the human body and which, additionally, make it possible to produce crystals suitable for a structure determination.

[0010] This object is accomplished by recombinant soluble Fc receptors which consist only of the extracellular portion of the receptor and are not glycosylated. The recep-

tors according to the present invention are therefore characterized by the absence of transmembrane domains, signal peptides and glycosylation.

[0011] Particularly preferred for the present invention are Fcγ or Fcε receptors. This is because IgG and IgE molecules are characteristic for a multiplicity of diseases and conditions, so that their determination and possible ways of influencing them are of great interest. FIGS. 11 and 12 show an alignment of amino acid sequences of the extracellular parts of some FcγRs and FcεRI. The FcRs according to the invention include all these sequences or parts thereof that still retain binding capacity to antibodies and/or proper crystallization.

[0012] In a particularly preferred embodiment of the invention the recombinant soluble FcR is a FcγRIIb receptor. Further, it is particularly preferred that the receptor be of human origin. In a particularly preferred embodiment, it contains an amino acid sequence as shown in one of SEQ ID NO:1 to SEQ ID NO:6.

[0013] According to the present invention, the preparation of the soluble Fc receptors preferably takes place in prokaryotic cells. After such expression, insoluble inclusion bodies containing the recombinant protein form in prokaryotic cells, thus facilitating purification by separation of the inclusion bodies from other cell components before renaturation of the proteins contained therein takes place. The renaturation of the FcRs according to the present invention which are contained in the inclusion bodies can principally take place according to known methods. The advantage of the preparation in prokaryotic cells, the production of inclusion bodies and the thus obtained recombinant soluble Fc receptors make it possible to obtain a very pure and, in particular, also very homogeneous FcR preparation. Also because of the absence of glycosylation the obtained product is of great homogeneity.

[0014] Soluble Fc receptors hitherto produced by recombinant means particularly exhibited the disadvantage that a much more elaborate purification was required, since they were expressed in eukaryotic cells and, due to the glycosylation which is not always uniform in eukaryotic cells, these products were also less homogeneous.

[0015] The recombinant soluble Fc receptors according to the present invention even make it possible to produce crystals suitable for use in X-ray analysis, as shall be explained later on in the description of further embodiments of the invention. The FcRs of the present invention moreover exhibit practically the same activity and specificity as the receptors naturally occurring in vivo.

[0016] A further subject matter of the present invention is a recombinant nucleic acid having a sequence coding for a recombinant soluble Fc receptor according to the present invention.

[0017] The nucleic acid according to the present invention may contain only the coding sequences or, additionally, vector sequences and/or, in particular, expression control sequences operatively linked to the sequence encoding the recombinant FcR, like promoters, operators and the like.

[0018] In a particularly preferred embodiment the nucleic acid of the present invention contains a sequence as shown in one of SEQ ID NO:7 to SEQ ID NO:12. For a compari-

son, SEQ ID NO:13 and SEQ ID NO:14 show the respective wild type sequences coding for FcγRIIb and FcεRIa. SEQ ID NOs:15-18 show the wild type sequences for FcγRI, FcγRIIa, FcγRIII and FcεRII.

[0019] If the nucleic acid of the present invention contains vector sequences, then these are preferably sequences of one or several prokaryotic expression vectors, preferably of pET vectors. Any other known functions or components of expression vectors may also be contained in the recombinant nucleic acid according to the present invention if desired. These may, for instance, be resistance genes allowing for an effective selection of transformed host cells.

[0020] A still further subject matter of the present invention is a host cell containing a recombinant nucleic acid according to the present invention. As repeatedly mentioned above, the host cell preferably is a prokaryotic host cell, particularly an *E. coli* cell.

[0021] The recombinant soluble Fc receptors according to the present invention can be used for a multitude of examinations or applications because they specifically react with antibodies. In vivo, the soluble Fc receptors are powerful immunoregulators which, if present in elevated levels, result in a remarkable suppression of the immune system which leads to many partly known and partly not yet understood effects. Based on these effects, several applications of the Fc receptors according to the present invention are further subject matters of the present invention.

[0022] One such subject is a process for the determination of the amount of antibodies of a certain type in the blood or serum of a patient, which is characterized by the use of a recombinant soluble FcR according to the invention in an immunoassay, and the determination of the presence of FcR-antibody complexes. Such assay allows to screen for the presence of a certain kind of antibody and allows also for the determination of the amount of antibodies present in the blood, plasma or serum of a patient.

[0023] Any type of immunoassay is principally suitable for the use according to the present invention, as long as the presence of FcR-antibody complexes can thereby be detected. Both ELISA (enzyme-linked immunosorbent immunoassay), particularly sandwich assays, and RIA (radio-immunoassay) are suitable, but also competitive testing methods. In a preferred embodiment of the invention where the presence and/or the amount of IgE antibodies is to be examined, an FcεR is used as recombinant soluble receptor according to the present invention. In particular, this method is suited and advantageous for determining a predisposition or manifestation of an allergy.

[0024] Moreover, a method is preferred in which the presence of soluble FcRs is to be determined and, if required, quantified. For such determination preferably a competitive immunoassay method is used, wherein as competition reagent a recombinant soluble receptor according to the invention is used, most preferably a recombinant FcγR. By means of this test among others the immune status of patients with chronic diseases of the immune system can be determined in a competitive immunoassay. Chronic diseases in the sense of these processes are for instance AIDS, SLE (systemic lupus erythematosus), MM (multiple myeloma) or rheumatoid arthritis, or in the case of FcεRII in B-CLL (Gordon et al., 1987), hyper IgE syndrome (Sarfati et al., 1988) or HCL (Small et al., 1990).

[0025] A further advantageous use of the recombinant receptor according to the present invention lies in the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

[0026] By means of modern screening techniques such as HTPS (high throughput screening) in combination with multi-well microtiter plates and automatic pipetting apparatuses it is nowadays possible to simultaneously test a multitude of substances for specific properties. As the FcRs according to the present invention can be easily produced at low cost, they can also be used in such series tests by which substances having an inhibiting effect can easily be identified.

[0027] Particularly preferred is such use according to which Fc receptors according to the present invention are used to find or screen inhibitors capable of inhibiting the recognition and binding of the respective antibodies to the particular receptor of interest.

[0028] A further area of application of the substances according to the invention lies in the pharmaceutical field. Hence, a further subject matter of the invention is a pharmaceutical composition comprising as active agent a recombinant soluble FcR according to the invention. According to the present invention, this pharmaceutical composition may of course comprise conventional useful carrier and auxiliary substances. Such substances are known to the person of skill in the art, the mode of administration also having to be taken into account. The pharmaceutical composition of the present invention can be advantageously used for the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

[0029] Soluble forms of Fc receptors such as FcγRIII mediate isotype-specific regulation of B cell growth and immunoglobulin production. In a murine model of myeloma, sFcR suppresses growth and immunoglobulin production of tumor cells (Müller et al., 1985; Roman et al., 1988; Teillaud et al., 1990). Furthermore, sFcR binds to surface IgG on cultures of human IgG-secreting myeloma cells and effects suppression of tumor cell growth and IgG secretion. Prolonged exposure of these cells to sFcR results in tumor cell cytotoxicity (Hoover et al., 1995).

[0030] Also, overreactions of the immune system in allergic reactions or due to massive antigen load might be reduced by, for example, intravenous application of soluble FcR (Jerino et al., 1993).

[0031] Therefore, a preferred pharmaceutical composition according to the invention for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma contains a recombinant soluble Fcγ receptor and, preferably, a receptor having the amino acid sequence as shown in SEQ ID NO:1-4.

[0032] It was also of great interest to obtain crystal structure data of Fc receptors and/or Fc receptor/Ig complexes. On the one hand, these are a key to the understanding of molecular mechanisms in immunocomplex recognition. On the other hand, these structural data can be used to find out common features in the structures of different Fc receptors and use the knowledge of the structures to generate inhibitors or identify and produce new artificial antibody receptors.

[0033] It was also of great interest to obtain information on the concrete binding sites of immunoglobulins to their respective receptors in naturally occurring three-dimensional molecules. Therefrom even more precise findings on the interactions between antibody and receptor can be obtained and also on how these interactions can be modulated. In this connection modulation means either an enhancement of the interaction or a reduction leading to an inhibition by e.g. covering the binding sites on one or more parts of the complex.

[0034] To obtain such crystal structure data and conformation information, a crystalline preparation of the recombinant soluble Fc receptor according to the invention is used. The recombinant soluble FcRs according to the invention surprisingly can be obtained pure enough to produce crystals that give reliable X-ray structure determination data. Such crystallization was not possible with the hitherto produced receptor molecules, mostly due to their lack of homogeneity.

[0035] Therefore, another embodiment of the present invention concerns a crystalline preparation of an Fc receptor according to the invention. Yet another embodiment of the present invention is a crystalline preparation of a complex of soluble Fc receptor according to the invention together with the related immunoglobulin Fc part. Particularly preferred embodiments are shown in the examples as well as the relevant crystal structure data. Via crystal structure analysis of the crystalline preparations the exact amino acids of the Fc receptor/Ig complexes could be detected which mediate the coupling. These amino acids are shown in FIGS. 6a and 6b and the type of binding between the individual amino acids of both molecules in the complex is also indicated. A further embodiment of the present invention is therefore the use of a crystalline preparation of a recombinant soluble Fc receptor for the generation of crystal structure data of Fc receptors. From this crystal structure data information about the three-dimensional structure and the active sites for the binding of antibodies can be obtained. Especially preferably is the use of a crystalline preparation of a complex of recombinant soluble Fc receptor according to the invention and the corresponding immunoglobulin molecule for the generation of crystal structure data for the complexes. These data allow to determine the actual interactions that are formed between the two molecules and allow for the first time to obtain exact information about the interaction of the molecules thereby conferring knowledge about possible sites for inhibition or enhancement of the binding. On the basis of the information obtained from the crystal structure data the findings necessary for effecting modulation of the interaction between Fc receptor and immunoglobulin can be obtained. This modulation can range from enhancement to complete inhibition to an inhibition of the binding.

[0036] The stated applications are merely preferred embodiments of the use of the crystal structure data. Many other applications seem possible, too.

[0037] Suitably, the structural data for the generation and/or identification of inhibitors or new receptors, respectively, are used in a computer-aided modelling program.

[0038] Particularly preferred for the present invention are the structures of FcRs or FcR:Fc-fragment complexes as exemplified in figures and examples. Such structures can be used to design inhibitors, antagonists and artificial receptor molecules.

[0039] Computer programs suitable for computer-aided drug design and screening are known to the person skilled in the art and generally available. They provide the possibility to examine umpteen compositions on the computer in view of their ability to bind to a certain molecule when the corresponding structure data are entered in the computer. With the help of this possibility a great number of known chemical compositions can be examined regarding their inhibiting or antagonistic effect. The person skilled in the art merely requires the crystal structure data provided by the present invention and a commercially available screening program (Program Flexx: From the GMD-German National Research Center for Information Technology, Schloss Birlinghoven, D-53754 Sankt Augustin, Germany). A preferred embodiment of the present invention therefore is the use of the crystal structure data obtained for the recombinant soluble Fc receptor according to the invention and for the complexes of recombinant soluble Fc receptor according to the invention and corresponding immunoglobulin in a computer aided modelling program for the identification and production of Fc receptor inhibitors.

[0040] Likewise, a further embodiment of the present invention is the use of the crystal structure data obtained for the receptors according to the invention and the receptor/immunoglobulin complexes, respectively for the identification and preparation of new Fc receptors which can be used, e.g. as antagonists and competitors. The crystal structure data and the data on the amino acids involved in the binding to Fc receptors obtained therefrom can serve for example to generate mutated immunoglobulins which can also be used as inhibitors. It is imaginable that mutated or chemically modified inhibitors undergo tight binding and thus effect a blocking of receptors. On the other hand, the data obtained for the binding sites of immunoglobulins can also be used for the identification and/or preparation of inhibitors for immunoglobulin molecules. Since the present invention teaches the binding sites to the receptor, it is easy to effect a blocking of the binding sites with the help of relatively simple molecules. Therefore, a further subject matter of the present invention is the use of the crystal structure data obtained for the FcR/Ig complexes for the identification and/or preparation of immunoglobulin inhibitors.

[0041] Accordingly, still further subject matter of the present invention are FcR inhibitors which have a three-dimensional structure which is complementary to the recombinant soluble FcR according to the invention and inhibit the binding of antibodies to FcRs.

[0042] Another further subject of the present invention are immunoglobulin inhibitors which have a three-dimensional structure which is complementary to the immunoglobulin binding site for recombinant soluble Fc receptors according to the invention and inhibit the binding of immunoglobulins to Fc receptors.

[0043] The term "complementary" is to be understood within the framework of the invention in such a way that the inhibitor molecules must be substances which are able to cover at least so many binding sites on the immunoglobulin or on the Fc receptor that the binding between Fc receptor and immunoglobulin is at least decisively weakened. Covering can take place both by binding to the amino acids mediating the complex formation of either component but also in such a way that at least complex formation is no

longer possible, be it by sterically inhibition or by binding to adjacent amino acids, however, covering the amino acid involved in the complex binding between Fc receptor and immunoglobulin.

[0044] In connection with the present invention it was possible for the first time to determine the exact binding sites and the amino acids involved in the binding of the antibody and antibody receptor molecules. One is now able to design specifically binding molecules and to screen candidate compositions on the computer. This enables the selection of such compositions from a variety of possibly candidate compositions which can effect a sufficient inhibition of complex formation between Fc receptor and immunoglobulin.

[0045] What is important for the inhibitors of the invention is that, owing to their structure and specificity, they are capable of binding to the FcRs or immunoglobulins and thus prevent the normal binding between FcRs and the constant parts of antibodies.

[0046] Preferably, such FcR or IgG inhibitors are small organic molecules which can easily be administered orally. They are an interesting alternative to cortisone in the treatment of autoimmune diseases and host/graft rejections. Such a molecule would also suppress reinfection rates with certain viruses, e.g. Dengue virus where the antibody coated virus is FcγRIIb dependent internalized (Littau et al., 1990), HIV where on CD4 positive T cells an antibody enhancement of HIV infection is mediated by FcγRIII (Homsy et al., 1989), or Ebola where the virus secreted glycoprotein inhibits early neutrophil activation by blocking sFcγRIII which affects the host response to infection (Yang et al., 1998).

[0047] The development of inhibitors also leads to substances that interfere with the recognition of IgE by their receptors. From the modelled structure of FcεRI, peptides have already been developed which inhibit mast cell degranulation in vitro. With the now available knowledge of the structures of the homologue receptors and the receptor-antibody complex in atomic detail, a new possibility for a rational drug design is opened.

[0048] The Fc-receptor bind between the two CH₂-domains of the Fc-fragment in the so-called lower hinge region (FIG. 8). The binding region of the Fc-receptor is described in Example 1 (The contact interface to IgG). The residues promoting the interaction between FcR and immunoglobulin are shown in FIGS. 7, 10a and 10b. Thereby three interaction regions become evident (FIG. 5).

1st Region: FcR (Residues 85 to 87 and Residue 110)-Ig (Chain A Residues 326-328)

[0049] Proline 328 of the Ig is clamped by the residues Trp 87 and 110 in a sandwich like manner. These residues are conserved among the IgG and IgE receptors as well as in the IgG and IgE. An inhibitor binding to this prominent region would strongly interfere with binding. This region is additionally attractive for inhibitor design because the exposed hydrophobic surface region comprising the residues Trp 87, Ile 85, Gly 86 of the receptors could be employed to obtain additional binding energy. The functional groups of Thr 113 and Glu 18 and Lys 19 side chains in the vicinity may contribute especially to specific inhibitor binding.

2nd Region: FcR (Residues 126-132 and Residues 155-158)-Ig (Chain A and Chain B Residues 234-239)

[0050] The amino terminal residues 234-239 of both Ig chains are recognised differently by the FcR, thereby breaking the 2-fold symmetry of the Fc fragment.

[0051] These residues of Fc-fragment chain A are in contact with residues Val 155-Lys 158 of the receptor and the same residues from Fc-fragment chain B with receptor residues Gly 126-His 132. This region shows the most differences in the sequence alignment of the receptors as well as the immunoglobulins and should therefore be involved in specificity generation. This deep cleft between the Fc-fragment chains is well suited for inhibitor design and would be the site of choice for the development of inhibitors when issues of specificity are concerned.

3rd Region: FcR (Residues 117, 126 and 129-132)-Ig (Chain B Residues 264-265 and Residues 296-297)

[0052] This binding region is characterised by a clustering of amino acid residues carrying functional groups in their side chains, that might be employed in various ways for inhibitor design on the receptor and the Ig side of the contact.

[0053] Molecules that interact with one or more of the above described regions, and are designed or screened explicitly for exploiting the knowledge of binding sites are considered as inhibitors according to the invention.

[0054] Further subject matters of the present invention are pharmaceutical compositions containing as active agent an FcR inhibitor or an immunoglobulin inhibitor as mentioned above. Such pharmaceutical compositions may, for example, be used in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system, preferably the treatment or prevention of allergies, autoimmune diseases or anaphylactic shock.

[0055] A further subject of the present invention is the sFcR according to the invention, bound to a solid phase. Such heterogeneous receptors can be used for immunoassays or other applications where the receptor in an immobilized form can be used beneficially.

[0056] In a preferred embodiment of the invention the solid phase is a chromatography carrier material onto which the Fc receptor is fixed, e.g. sepharose, dextran sulfate etc. Such chromatography materials with Fc receptors bound thereto can beneficially be used for the adsorption of immunoglobulins from the blood, plasma or serum of patients or from the culture supernatant of immunoglobulin producing cells (meaning concentration, enrichment and purification of antibodies).

[0057] On the one hand, the antibodies bound to the chromatography material can be eluted and, for example, the immune status of a patient can thereby be determined. On the other hand, antibodies from the blood of a patient can thereby be enriched before carrying out further tests, which is a further preferred embodiment of the present invention. In many cases it is difficult to conduct diagnostic assays using blood samples if the latter contains only a very small number of the antibodies to be identified. By means of a concentration using a specific chromatographic column with Fc receptors according to the present invention, antibodies

of interest can easily be concentrated and separated from many other substances which might disturb the test.

[0058] Basically, it is also possible to use a chromatography material according to the present invention in an extracorporeal perfusion system for lavage of the blood in case of certain diseases where the removal of antibodies plays a crucial role.

[0059] It is, however, also possible to use another material as solid phase to which the soluble Fc receptor according to the invention is coupled, e.g. microtiter plates or small reaction vessels to the walls of which Fc receptors are bound either directly or indirectly. Such solid phases and vessels can be particularly important for diagnostic methods, as they enable screening by using immunoassays e.g. for detecting the presence of certain immunoglobins in patients' blood or other body fluids.

[0060] To sum up, the recombinant soluble Fc receptors provided by the present invention as well as the corresponding structure determination of crystalline preparations of these receptors and of crystalline complexes of receptors and immunoglobins enable for the first time to perform a rational drug design, wherefrom it is possible to modulate the interaction between immunoglobulins and Fc receptors on cells or soluble receptors. Such a modulation is preferably an inhibition, whereby the inhibition of the formation of a complex from IgG and Fc receptor takes place by covering and preferably by binding of inhibitor molecules to the Fc receptor or the immunoglobulin. There are various medical applications for such modulating drugs and in particular of inhibitors and only few of these applications have been exemplarily mentioned within the framework of the present specification. This can and should by no means exclude the applicability of such molecules which have been designed or screened on the basis of the findings about the molecular structure or FcR/Ig complexes disclosed herein for the treatment or prevention of other health disturbances.

[0061] The following Examples are to further illustrate the invention in conjunction with the Figures.

EXAMPLE 1

shFcγRIIb (soluble human FcγRIIb)

1.1 Cloning and Expression

[0062] The cDNA of human FcγRIIb2 (Engelhardt et al., 1990) was modified using mutagenous PCR (Dulau et al., 1989). Therefore, a forward primer was used for the introduction of a new start methionine after the cleavage site of the signal peptide within a NcoI site (5'-AAT AGA ATT CCA TGG GGA CAC CTG CAG CTC CC-3') while the reverse primer introduced a stop codon between the putative extracellular part and the transmembrane region followed by a SalI site (5' CCC AGT GTC GAC AGC CTA AAT GAT CCC C-3'). The PCR product was digested with NcoI and SalI, cloned into a pET11d expression vector (Novagen) and the proposed sequence was confirmed. The final construct was propagated in BL21 (DE3) (Grodberg and Dunn, 1988). For the overexpression of FcγRIIb a single colony of the transformed bacteria was inoculated in 5 ml LB medium containing 100 µg ampicillin per ml (LB-Amp100) and incubated overnight at 37° C. The culture was diluted 200-fold in LB-Amp100 and incubation was continued until an OD600 of 0.7-0.9 was achieved. The overproduction of

the protein was induced by adding IPTG to a final concentration of 1 mM. After a growing period of 4 hours the cells were harvested by centrifugation (30 min, 4000×g) and resuspended in sonification buffer (30 mM sodium phosphate, 300 mM sodium chloride, 0.02% sodium azide, pH 7.8). After addition of 0.1 mg lysozyme per ml suspension and incubation for 30 min at room temperature the sonification was performed on ice (Branson Sonifier, Danbury, Conn.; Macrotip, 90% output, 80% interval, 15 min). The suspension was centrifuged (30 min, 30,000×g) and resuspended with a Dounce homogenizer in sonification buffer containing 0.5% LDAO. The centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The purified inclusion bodies were stored at 4° C.

1.2 Refolding and Purification of Soluble Human FcγRIIb (shFcγRIIb)

[0063] The purified inclusion bodies were dissolved to a protein concentration of 10 mg/ml in 6 M guanidine chloride, 100 mM 2-mercaptoethanol and separated from the insoluble matter by centrifugation. The refolding was achieved by rapid dilution. Therefore, one ml of the inclusion body solution was dropped under stirring within 15 hours into 400 ml of the refolding buffer (0.1 M TRIS/HCl, 1.4 M arginine, 150 mM sodium chloride, 5 mM GSH, 0.5 mM GSSG, 0.1 mM PMSF, 0.02% sodium azide, pH 8.5, 4° C.). Afterwards, the mixture was stirred for 2-3 days until the concentration of free thiol groups was reduced to 1 mM by air oxidation as measured according to Ellman (Ellman, 1959). The solution was dialyzed against PBS and sterile filtered before it was concentrated 10-fold in a stirring cell equipped with a 3 kD MWCO ultrafiltration membrane. The protein solution was applied to a hIgG sepharose column (50 mg hIgG per ml sepharose 4B). Unbound protein washed out with 50 mM TRIS pH 8.0 before elution of FcγRIIb by pH jump (150 mM sodium chloride, 100 mM glycine, 0.02% sodium azide, pH 3.0). The eluate was immediately neutralized with 1 M TRIS pH 8.0. The FcγRIIb containing solution was concentrated and subjected to gel filtration on a Superdex-75 column equilibrated with crystallization buffer (2 mM MOPS 150 mM sodium chloride, 0.02% sodium azide pH 7.0). The fractions containing FcγRIIb were pooled, concentrated to 7 mg/ml and stored at -20° C.

1.3 Equilibrium Gel Filtration Experiments

[0064] A Superdex75 column was connected to FPLC and equilibrated with PBS containing 10 μg shFcRIIb per ml. Human Fc fragment was solved to a concentration of 1 μg/10 μl in the equilibration buffer and injected. The resulting chromatogram yielded a positive peak comprising the complex of the shFcγRIIb and the Fc fragment while the negative peak represents the lack of receptor consumed from the running buffer for complex formation.

1.4 Crystallization and Data Collection

[0065] Initial crystallization trials employing a 96 condition sparse matrix screen (Jancarik and Kim, 1991) were performed in sitting drops at 20° C. using the vapor diffusion method. Occurring crystals were improved by changing the pH as well as the salt, precipitant and additive concentration. Diffraction data from suitable crystals 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 (Leslie, 1997) and subsequently the data was scaled, reduced and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

1.5 Summary of Expression, Purification and Refolding of shFcγRIIb

[0066] The extracellular part of FcγRIIb was expressed in high levels under the control of a T7 promoter in the T7 RNA polymerase positive *E. coli* strain BL21/DE3 (Grodberg & Dunn, 1988). The protein was deposited in inclusion bodies, which were employed in the first purification step. The isolation of the inclusion bodies was started with an intense combined lysozyme/sonification procedure to open virtually all cells which would otherwise contaminate the product. The subsequent washing steps with the detergent LDAO, which has excellent properties in solving impurities but not the inclusion bodies itself already yielded a product with a purity of >90% (FIG. 1).

[0067] This product was used for refolding trials without further purification. The inclusion bodies were dissolved in high concentration of 2-mercaptoethanol and guanidine to ensure the shift of covalent and non-covalent aggregates to monomers. This solution was rapidly diluted with refolding buffer to minimize contacts between the unfolded protein molecules which would otherwise form aggregates. The use of arginine in the refolding buffer prevents the irreversible modification of side chains as often recognized with urea. After addition of the protein to the refolding buffer, the solution was stirred at 4° C. until the concentration of free thiol groups was reduced to 1 mM, which was absolutely necessary as earlier dialysis resulted in an inactive product. In a second purification step the dialyzed and refolded FcγRIIb was bound to immobilized hIgG to remove minor fractions of *E. coli* proteins and inactive receptor. The protein was eluted with a pH jump and immediately neutralized. After this affinity chromatography step shFcγRIIb is essentially pure except for a minor contamination resulting from the coeluting IgG which leached out of the matrix even after repeated use (FIG. 1). The IgG as well as receptor multimers which are not visible in the reducing SDS-PAGE could easily be removed by gel filtration. Parallel to the removal of the contaminants in this step the buffer is quantitatively exchanged. This procedure ensures a defined composition of the protein solution as even slight variations can cause irreproducibility of the crystallization attempts or even inhibit the formation of crystals. Overall 6 mg pure protein could be gained per litre *E. coli* culture, which is about 10% from the FcγRIIb content of the inclusion bodies.

[0068] N-terminal protein sequencing revealed the identity with the expected sequence H₂N-GTPAAP without detectable contamination. ESI-MS analysis showed that the final material used in crystallization trials is homogenous with respect to size. From the primary sequence the molecular weight was calculated to 20434 Da, which corresponds to 20429 Da found by mass spectroscopy. The discrepancy lies within the error of the instrument, and no additional peak for a species containing the leading methionine is found.

[0069] The crystallization of shFcγRIIb was performed in sitting drops using the vapor diffusion method. Initial trials

with a sparse matrix screen (Jancarik & Kim, 1991) resulted already in small crystalline needles. Subsequent optimization of the preliminary crystallization condition by varying precipitant, salt, their concentration and pH led to the isolation of three different crystal forms. Orthorhombic crystals grew from mixture of 1.5 μ l reservoir solution (33% PEG2000, 0.2 M sodium acetate, pH 5.4) with 3 μ l of the protein solution. They appeared within 3 days and reached their final size of approximately 80 μ m \times 80 μ m \times 500 μ m after one week. These crystals diffracted to 1.7 Å. Crystals could also be grown in two other space groups from reservoir solution containing 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5 mM Zn(OAc)₂, 100 mM sodium chloride (hexagonal form) and 26% PEG8000, 0.2 M NaOAc, pH 5.6, 10% (v/v) 1,4-Dioxan, 100 mM sodium chloride (tetragonal form). These crystals were of suitable size for X-ray analysis but diffracted only to 2.7 Å and 3.8 Å for the tetragonal and hexagonal crystal form respectively (Table 1).

[0070] FcγRII was expressed in *E. coli* which, besides the comparatively low production costs and the availability, has several advantages especially when the glycosylation performed by mammalian cells is not necessary for the function of the protein as in the case of FcγRII where IgG binding occurs independently of carbohydrate attachment (Sondermann et al., 1998A). In *E. coli* a homogenous product can reproducibly be generated, which is in contrast to the expression in mammalian cells where batch dependent variances are often observed. In such a system the product is for several days exposed to proteases at temperatures of more than 30° C. In contrary, the expression of the protein in *E. coli* under the control of the strong T7 promoter at 37° C. frequently leads to the formation of protease inaccessible inclusion bodies. A further advantage of the expression in bacteria is that the material could be considered to be free of pathogenic germs, which might derive from employed fetal calf serum or the cell line itself. In mammalian expression particular care must be taken during the purification of the target protein because potential effective hormones or growth factors might be copurified. One case where the effects of sFcγR were ascribed to a TGFβ1 contamination is already reported (Galon et al., 1995).

1.6 Purification

[0071] The purification procedure is straightforward. It consists of three steps which can easily be performed in a single day. The protein is obtained in a pure form and in high yields and could even be obtained in considerable quality without the expensive IgG affinity column. The success of such a protocol would depend on the careful preparation of the inclusion bodies, as most of the impurities can be eliminated already in the first purification step.

1.7 Characterization

[0072] The purified FcγRIIb was characterized by SDS-PAGE and isoelectric focussing as well as N-terminal sequencing and mass spectroscopy. Thus, the material can be considered pure and homogeneous with respect to its chemical composition, but the intriguing question whether the receptor is correctly folded remains to be discussed. All cysteins are paired, since no free thiol groups are detected with Ellman's test. The material is monomeric and eludes with the expected retention time in peaks of symmetrical shape from a size exclusion chromatography column. Fur-

thermore, FcγRIIb binds to IgG sepharose, recombinant FcγRIIb from *E. coli* is active because it specifically binds IgG.

1.8 Crystallization

[0073] The orthorhombic crystal form of FcγRIIb diffracted X-rays to a resolution of 1.7 Å, which is a drastic improvement compared to previously reported crystals of the same molecule derived from insect cell expression (Sondermann et al., 1998A). These crystals diffracted to 2.9 Å and were of space group P3₁21. Thus, the glycosylation of the insect cell derived receptor influences the crystallization conditions. Instead of the trigonal space group, three different crystal forms are found. After a possible solution of the structure these crystal forms will help identify artificial conformations of the protein due to crystal contacts.

[0074] FcγRs do not exhibit any sequence similarity to other proteins but due to a conserved cystein spacing they are affiliated to the immunoglobulin super family. Consequently, we tried to solve its structure by molecular replacement, but extensive trials using IgG domains from a variety of molecules failed. Thus the structure of FcγRIIb has to be solved by the methods of multiple isomorphous replacement.

[0075] We have shown for the first time that FcγRIIb can be obtained in an active form from *E. coli*. This is the basis for crystallographic investigations that will soon, due to the already gained crystals of exceptional quality, result in the structure solution of this important molecule. The structure will provide information on the IgG binding site and provide a starting point for the knowledge based design of drugs that interfere with recognition of the ligand by its receptor. Furthermore, because of the high homology between FcγRIIb and other FcRs including FcεRIa it seems possible that these molecules can be produced in the same way, which would provide valuable material for the ongoing research.

1.9 Methods

Protein Chemistry

[0076] Recombinant soluble human FcγRIIb was expressed in *E. coli*, refolded purified and crystallized as described elsewhere (Sondermann et al., 1998B). 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 \times g) and washed with buffer containing 0.5% LDAO. A centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The inclusion bodies were solved in 6 M guanidine hydrochloride and the protein was renatured as described. The dialyzed and filtrated 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

[0077] Crystallization was performed in sitting drops at 20° C. using the vapor diffusion technique. Crystallization screens were performed by changing pH, salt, precipitant and additives. The final crystals used for data collection

were grown in 33% PEG2000, 0.2 M sodium acetate, pH 5.4 (orthorhombic form) 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 10% (v/v) 1,4-dioxane, 100 mM sodium chloride (tetragonal form), and 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5 mM ZN(OAc)₂, 100 mM sodium chloride (hexagonal form). The insect cell derived protein was crystallized in 32% PEG6000, 0.2 M sodium acetate, pH 5.3.

Preparation of Heavy Atom Derivatives

[0078] 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 hours or 10 mM uranylchloride for 8 days.

X-Ray Data Collection

[0079] 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 subsequently the data was scaled and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

Structure Determination

[0080] 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 the 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 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 2. 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 Incorporation). 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 (Engh & Huber, 1991). When the structure was complete after several rounds of model building and individual restraint B-factors refinement ($R_{\text{fac}}=29\%/R_{\text{Free}}=36\%$), 150 water molecules were built into the electron density when a Fo-Fc map contoured at 3.5 σ coincided with well defined electron density of a 2Fo-Fc map contoured at 1 σ . The resulting refinement statistic is shown in Table 3.

1.10 Structure Determination

[0081] 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, PDB entry 1fc1; Deisenhofer, 1981) failed. The putative extracellular part of the receptor (amino acid residues 1-187 as

depicted in SEQ ID NO:2) was used for crystallization trials (Sondermann et al., 1998B) while the model contains the residues 5-176 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 2. The structure contains a cis proline at position 11. None of the main chain torsion angles is 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 P4₂2₁2 and of the glycosylated form derived from insect cells in crystals of space group P3₁21 (Table 2).

[0082] The polypeptide chain of FcγRIIb folds into two Ig-like domains as expected from its affiliation with the immunoglobulin super family. Each domain consists of two beta sheets that are arranged in a sandwich with the conserved disulfide bridge connecting strands B and F on the opposing sheets (FIG. 3). Three anti-parallel β-strands (A1, B, E) oppose a sheet of 5 β-strands (C', C, F, G, A2), whereby strand A1 leaves the 3-stranded β-sheet and crosses over to the 4-stranded anti-parallel sheet adding the short parallel 5th strand A2. 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 a r.m.s. distance of 1.29 Å of 67 matching Ca atoms.

[0083] The domains are arranged nearly perpendicularly to each other enclosing an angle of 70 degrees between their long axes forming a heart-shaped overall structure. This arrangement results in an extensive contact region between the domains (FIG. 4). Residues from strand A2 and from the segment linking A2 and A1 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. This is confirmed by the conservation of the structure in three different space groups. In orthorhombic, tetragonal and hexagonal (insect cell derived) crystal forms a deviation of less than 20 in the interdomain angle is found.

1.11 Overall Structures

[0084] The structure of recombinant human FcγRIIb derived from *E. coli* was solved by MIR to 1.7 Å resolution from orthorhombic crystals. An essentially identical structure is found in tetragonal 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 to allow some reorientation of the receptor to enhance the recognition of the Fc parts in immunocomplexes.

1.12 Homologue Receptors

[0085] The Ig domains found in the Ig super family of proteins are characterized by a beta sandwich structure with a conserved disulfide bridge connecting two strands of the opposing sheets. The typical arrangement of 3 and 4 anti parallel beta strands that form a sandwich as found in FcγRIIb occurs also in the T cell receptor, Fc fragment, CD4 or the Fab fragment. A structural alignment of the individual Ig domains of these molecules with the two domains of

FcγRIIb shows a common, closely related structure. The relative arrangement of the domains, however, is not related in these molecules and covers a broad sector. Despite the structural similarity between Ig domains from different molecules and the strikingly low r.m.s. deviation of Cα atoms that result when the two domains of FcγRII are superimposed, no significant sequence similarity is found (FIGS. 5a and 5b). A structure-based sequence alignment shows a conserved hydrophobicity pattern along the sequence of the domains, together with, beside the cysteins, 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 FcγRI and the 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. Firstly, the different domains of an FcR are more related to each other than to Ig domains from other molecules of the Ig super family. Secondly, the N-terminal domains of the receptors relate to each other as the second domains do. Thirdly, the sequence of the third domain of FcγRI shows features from both groups of domains. Taken together, we confirm the affiliation of the FcRs to the Ig super family and speculate that all FcR-domains originate from a common ancestor, an ancient one 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 that acquired a third domain.

[0086] Conservation of these amino acid residues that contribute to the interdomain contact in FcγRIIb in the alignment are a hint to a similar domain arrangement in different receptors. In Table 4 the residues contributing with their side chains to the interdomain contact (FIG. 4) are compiled for FcγRIIb together with the corresponding amino acid residues in other receptors according to the structure-based sequence alignment of FIG. 5b. Except for Asn15, which is not conserved between the FcRs, the involved residues are identical or conservatively replaced providing strong support for a similar structure and domain arrangement in all FcRs.

1.13 The Contact Interface to IgG

[0087] Limited information about the interactions of FcRs with their ligands is available from mutagenesis studies (Hogarth et al., 1992; Hulett et al., 1994; Hulett et al., 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 (FIG. 3, FIG. 5b). 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 Arg 131 to H is 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 Pro 114, Leu 115 and Val 116 in the neigh-

borhood of Tyr 157. This patch is separated from the region Leu 159, Phe 121 and Phe 129 by the positively charged amino acid residues Arg 131 and Lys 117 which protrude from the core structure (FIG. 5b).

1.14 Glycosylation

[0088] 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 (FIG. 3, FIG. 6). Since the material used for the solution of this structure was obtained from *E. 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 glycosylation in binding. This was confirmed by the structure of the FcγRIIb produced in insect cells which is glycosylated (Sondermann et al., 1998A). Except for a 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 4 bands. No additional electron density for those sugars was found a consequence of chemical and structural heterogeneity.

EXAMPLE 2

shFcγRIIa (Soluble Human FcγRIIa)

[0089] The procedures were performed according to example 1 except for the indicated changes:

2.1 Cloning and Expression

[0090] shFcγRIIa was generated by mutating the respective wild-type cDNA (Stengelin et al., 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET22b+ vector was chosen.

2.2 Refolding and Purification

[0091] shFcγRIIa was refolded according to example 1 with the respective refolding buffer listed in table 6.

2.3 Crystallisation

[0092] shFcγRIIa was crystallised as described under conditions indicated in table 7.

2.4 Structure Determination

[0093] The structure was solved with the method of isomorphous replacement with shFcγRIIb as search model.

EXAMPLE 3

shFcγRIII (Soluble Human FcγRIII)

[0094] The procedure was performed according to example 1 except for the indicated changes:

3.1 Cloning and Expression

[0095] shFcγRIII was generated by mutating the respective wild-type cDNA (Simmons & Seed, 1988) and

expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET22b+ vector was chosen.

3.2 Refolding and Purification

[0096] shFcγRIII was refolded according to example 1 with the respective refolding buffer listed in table 6.

3.3 Crystallisation

[0097] shFcγRIII was crystallised as described under conditions indicated in table 7.

3.4 Structure Determination

[0098] The structure was solved with the method of isomorphous replacement with shFcγRIIb as search model.

3.5 Crystallisation of a shFcγRIII:hFc1 complex

[0099] hIgG1 derived from the serum of a myeloma patient was used to prepare Fc-fragments (hFc1) by digestion with plasmin (Deisenhofer et al., 1976). The resulting Fc-fragments were separated from the Fab-fragments by protein A chromatography. Partially digested hIgG was removed by size exclusion chromatography with MBS (2 mM MOPS, 150 mM NaCl, 0.02% sodium azide, pH 7.0) as running buffer. Equimolar amounts of hFc1 and shFcγRIII were mixed and diluted with MBS to a concentration of 10 mg/ml. The complex was crystallised as described under conditions indicated in table 5.

EXAMPLE 4

shFcεRII (Soluble Human FcεRII)

[0100] The procedure was performed according to example 1 except for the indicated changes:

4.1 Cloning and Expression

[0101] FcεRII was generated by mutating the respective wild-type cDNA (Kikutani et al., 1986) and expressed according to example 2 with the mutagenous primers listed in table 5. For the expression of the protein a pET23a+ vector was chosen.

4.2 Refolding and Purification

[0102] Refolding of shFcεRII was achieved as described in example 1, with the exception that prior to rapid dilution the dissolved inclusion bodies were dialysed against 6M guanidine chloride, 20 mM sodium acetate, pH 4.0. shFcεRII was refolded according to example 1 with the respective refolding buffer listed in table 6. After refolding the protein solution was dialysed against PBS, concentrated 100-fold and purified by gel filtration chromatography on Superdex 75. This yielded pure shFcεRII which was dialysed against 2 mM TRIS/HCl, 150 mM NaCl, 0.02% sodium azide, pH 8.0, concentrated to 10 mg/ml and stored at 4° C.

EXAMPLE 5

[0103] shFcγRI (Soluble Human FcγRI)

[0104] The procedure was performed according to example 1 except for the indicated changes:

5.1 Cloning and Expression

[0105] shFcγRI was generated by mutating the respective wild-type cDNA (Allen & Seed, 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET32a+

vector was chosen, which contains after the N-terminal thioredoxin a hexahistidine-tag with a C-terminal thrombin cleavage site followed by the shFcγRI in frame with the mentioned proteins and amino acid residues. For the over-expression of the fusion protein the *E. coli* strain BL21 (DE3) containing the plasmids pUBS and pLysS (Novagen) was used.

[0106] The purified inclusion bodies were solubilised in 6M guanidine-HCl, 10 mM β-mercaptoethanol, 50 mM Tris pH8.0 and bound to a Ni-NTA column (Qiagen). The elution was performed with an imidazole gradient ranging from 0 to 1 M imidazole. The eluted protein was dialysed against a 1000 fold volume of 150 mM NaCl, 50 mM Tris pH8.0, 2 mM GSH, 0.5 mM GSSG for 24 hours at 4° C. After concentrating the protein solution to 25% of the initial volume, thrombin was added. After 6 h of incubation at 37° C. the N-terminal thioredoxin and the His-tag were removed completely as verified by N-terminal sequencing. During this digestion the shFcγRI precipitated quantitatively out of solution.

5.2 Refolding and Purification

[0107] shFcγRI was refolded according to example 1 with the respective refolding buffer listed in table 6. After the redox potential decreased to 1 mM the solution was dialysed against PBS pH8.0 and concentrated.

[0108] The refolded Protein was analysed by size exclusion chromatography, which yielded a peak of the proposed monomeric receptor and non reducing SDS-PAGE which showed a major band at 30 kDa.

EXAMPLE 6

shFcεRIa (Soluble Human FcεRIa)

[0109] The procedure was performed according to example 1 except for the indicated changes:

6.1 Cloning and Expression

[0110] shFcεRI was generated by mutating the respective wild-type cDNA (Kochan et al., 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET23a+ vector was chosen.

BRIEF DESCRIPTION OF THE FIGURES

[0111] FIG. 1: 15% reducing SDS PAGE showing the purification of sFcγRIIb

[0112] Lane 1: Molecular weight marker. Lane 2: *E. coli* lysate before induction. Lane 3: *E. coli* lysate 1 h after induction. Lane 4: *E. coli* lysate 4 h after induction. Lane 5: Purified inclusion bodies of sFcγRIIb. Lane 6: Eluate of the hIgG affinity column. Lane 7: Pooled fractions of the gel filtration column.

FIG. 2: Equilibrium gel filtration

[0113] 1 μg hFc solved in 10 μl equilibration buffer (10 μg sFcγRIIb/ml PBS) was applied to a size exclusion chromatography column and the absorbance of the effluent was measured (280 nm) as a function of time. The injected Fc fragment forms a complex with the sFcγRIIb in the equilibration buffer (t=22 min). The negative peak of consumed sFcγRIIb is observed at t=26 min.

FIG. 3: Overall structure of human sFcγRIIb

[0114] 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 labeled 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. 4: Interdomain contacts

[0115] The figure shows a close-up on 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 2Fo-Fc electron density contoured at 1 σ 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).

[0116] FIG. 5a: Superposition of the two FcγRIIb domains and the CH2 domain of human IgG1

[0117] 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 labeled and the conserved disulfide bridges are depicted as thin lines.

[0118] FIG. 5b: Structure based sequence alignment of the sFcγRIIb domains with domains of other members of the FcR family

[0119] 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 & Schomburg, 1994). Amino acid residues with a Cα distance of less than 2.0 Å in the superimposed domains are masked: lilac for matching residues between the Fc fragment domains; yellow for 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 labeled consistent with FIG. 3.

[0120] 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 cysteins are typed 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).

[0121] FIG. 6: The putative binding sites of FcγRIIb

[0122] Solid surface representations of FcγRIIb as produced with GRASP (Nicholls et al., 1991), the color coding is according to the relative surface potential from negative

(red) to positive (blue). FIG. 6a shows the molecule as in FIG. 3 by a rotation of about 90° counter-clockwise around the vertical. In FIG. 6b the molecule is rotated 90 clockwise around the same axis. Both views show the putative binding regions on the C-terminal (FIG. 6a) and the N-terminal domain (FIG. 6b). The amino acid residues discussed in the text are labeled.

[0123] FIG. 7: Cα-trace of the superpositioned structures of the Fcγ-receptors FcγRIII red, FcγRIIa green and FcγRIIb blue. Residues important for IgG binding are shown in ball-and-stick. The N- and C-termini are labelled.

[0124] FIG. 8: Overview of the FcγRIII/Fc-fragment crystal structure in ribbon representation

[0125] The sugar residues bound to the Fc-Fragment are indicated in ball-and-stick. The FcγRIII (blue) binds in the lower hinge region between chain-B (red) and chain-A (green) of the Fc-fragment.

[0126] FIG. 9: Close-up on the binding region of the FcγRIII and the Fc-fragment

[0127] The colour scheme is in agreement to FIG. 8 and residues important for complex formation are shown in ball-and-stick.

[0128] FIG. 10a:

[0129] In the upper part of FIG. 10a a structure based sequence alignment of the Fc-Receptor ecto-domains is shown. Conserved residues are shaded yellow and identical residues orange. The lower part of the figure shows a part of the alignment of human antibody sequences. Residues of the human FcγRIII in contact with the Fc-fragment in the complex crystal structure are connected by lines (black for hydrophobic interaction, red for salt bridges and blue for hydrogenbridges). Residues from the Fc-receptor in contact with the A-chain of the Fc-fragment are connected with dashed lines and those in contact with the B-chain of the Fc-fragment with solid lines. Red, blue and black lines represent charged, polar and other contacts, respectively.

[0130] FIG. 10b:

[0131] In the upper part of FIG. 10b a structure based sequence alignment of the Fc-Receptor ecto-domains is shown. Conserved residues are shaded yellow and identical residues orange. Conserved residues within the less related Kir and FcA-Receptor sequences are shaded blue.

[0132] The lower part of the figure shows a part of the alignment of human antibodies with the mouse IgE (mIgE) sequence. Residues of the human FcγRIII in contact with the Fc-fragment in the complex crystal structure are connected by lines (black for hydrophobic interaction, red for salt bridges and blue for hydrogenbonds). Residues from the Fc-receptor in contact with the A-chain of the Fc-fragment are connected with dashed lines and those in contact with the B-chain of the Fc-fragment with solid lines. Red, blue and black lines represent charged, polar and other contacts, respectively.

[0133] FIG. 11 and FIG. 12:

[0134] FIG. 11 and FIG. 12 show an alignment of the produced sFcγR, sFcεRIa and the short form of sFcεRII and the produced sFcγR and sFcεRIa without sFcεRII, respectively.

TABLE 1

Crystallographic results			
The obtained preliminary crystallographic data are shown in this table.			
	Orthorhombic	Tetragonal	Hexagonal
Space group	P2 ₁ 2 ₁ 2 ₁ [19]	P4 ₂ 2 ₁ 2 [94]	P3 [143]
Unit cell dimensions	a = 40.8 Å, b = 50.9 Å, c = 80.5 Å, α = 90°, β = 90°, γ = 90°	a = 85.7 Å, b = 85.7 Å, c = 63.4 Å, α = 90°, β = 90°, γ = 90°	a = 80.9 Å, b = 80.9 Å, c = 157.0 Å, α = 90°, β = 90°, γ = 90°
R _{merge}	5.8%	9.8%	13.6%
Resolution	1.7 Å	2.7 Å	3.8 Å
Unique	18,040	6,616	7,210
Completeness	89.1%	97.1%	63.0%
Multiplicity	3.5	4.4	1.3
V _M , molecules per asymmetric unit, solvent content	2.09 Å ³ /Da, 1 mol., 41% solvent	2.91 Å ³ /Da, 1 mol., 58% solvent	2.97 Å ³ /Da, 5 mol., 59% solvent

[0135]

TABLE 2

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 ₁ 2 ₁ 2 ₁	18009	3.6	1.74	92.9/86.4	5.5		
NATI	P4 ₂ 2 ₁ 2	6615	4.5	2.70	97.1/94.3	10.1		
NATI-Baculo	P3 ₁ 21	3545	2.5	3.0	93.0/98.9	14.4		
UOAc	P2 ₁ 2 ₁ 2 ₁	7722	4.2	2.1	96.8/95.7	7.3	1	1.79
PtPγ	P2 ₁ 2 ₁ 2 ₁	5520	3.9	2.3	89.7/49.6	10.5	1	1.39

$$R_m = \sum |I_h - \langle I_h \rangle| / \sum \langle I_h \rangle$$

Phasing power: $\langle F_H \rangle / E$, where $\langle F_H \rangle = \sum (F_H^2/n)^{1/2}$ is the r.m.s. heavy atom structure amplitude.

$E = \sum [(F_{PHC} - F_{PH})^2/n]^{1/2}$ is the residual lack of closure error with F_{PH} being the structure factor amplitude and $F_{PHC} = IF_P + F_H I$ the calculated structure factor amplitude of the derivative.

[0136]

TABLE 3

Refinement statistics	
Resolution range (Å)	8.0-1.74 Å
No. of unique reflections (F > 0σ (F))	16252
R factor	19.4
R _{free} *	27.9
No. of atoms per asymmetric unit	
protein	1371
solvent	150
Rms 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

TABLE 3-continued

Refinement statistics	
solvent	36.7
Rms deviation of bonded B factors (Å ²)	4.1

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

[0137]

TABLE 4

Residues that contribute to the interdomain contact via side chains				
FcyRIIb	FcyRIIIa	FcyRIII	FcyRI	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

[0138]

TABLE 5

Primers used for the amplification of the FcRs		
Construct	5'-Primer	3'-Primer
sFcγRI	5'- <u>CACCCATATG</u> GCAGTGATCTCTTT-3'	5'-AGGACTCGAGA CTAGACAGGAGTTGGTAAC-3'
sFcγRIIa	5'-ACAGTCATATG GCAGCTCCCC-3'	5'-AAAAAAGCTz, 803 GGGCACTTGGAC-3'
sFcγRIIb	5'-AATTCCTATGGGACACCTGCAGCTCCC-3'	5'-CCCAGTGTGACAG CCTAAATGATCCCC-3'
sFcγRIII	5'-AAAAAACATATGCGGACTGAAG-3'	5'-AAAAAAGCTTAACTTGAGTGATG-3'
sFcεRIa	5'-GATGGCCATATGGCAGTCCCTCAG-3'	5'-CAATGGAT CCTAAAATTGTAGCCAG-3'
sFcεRII	5'-AAAAAACATATGGAGTTGCAGG-3'	5'-TGGCTGGATCCATGCTCAAG-3'

Introduced restriction sites are underlined, start- and stop-codons are depicted as bold-italics

[0139]

TABLE 6

Refolding Conditions for the FcRs	
Construct	Buffer
sFcγRI	0.1M TRIS/HCl, 1.2M arginine, 150 mM NaCl, 5 mM GSH, 0.5 mM GSSG, 0.02% sodium azide, pH 8.0
sFcγRIIa	0.1M TRIS/HCl, 1.4M arginine, 150 mM NaCl, 2 mM GSH, 0.5 mM GSSG, 0.02% sodium azide, pH 8.0
sFcγRIIb	0.1M TRIS/HCl, 1.4M arginine, 150 mM NaCl, 5 mM GSH, 0.5 mM GSSG, 0.02% sodium azide, pH 8.0
sFcγRIII	0.1M TRIS/HCl, 1.0M arginine, 150 mM NaCl, 2 mM GSH, 0.5 mM GSSG, 0.02% sodium azide, pH 8.0
sFcεRII	0.1M TRIS/HCl, 0.8M arginine, 150 mM NaCl, 5 mM GSH, 0.5 mM GSSG, 0.02% sodium azide, pH 8.3

[0140]

TABLE 7

Crystallisation Conditions for the FcRs			
Construct	Condition	Space group, cell constants	Resolution
sFcγRIIa	26% PEG 8000, 0.2M sodium acetate/acetic acid pH 4.6, 0.02% sodium azide	C2, a = 80.4 Å, b = 49.7 Å, c = 54.6 Å, a = g = 90°, b = 128.1°	3.0 Å
sFcγRIIb	33% PEG 2000, 0.2M sodium acetate, 0.02% sodium azide, pH 5.4	P212121, a = 40.8 Å, b = 50.9 Å, c = 80.5 Å, a = b = g = 90°	1.7 Å
sFcγRIII	22% PEG 8000, 0.1M MES/TRIS pH 7.8, 0.02% sodium azide	P22121, a = 36.7 Å, b = 60.3 Å, c = 85.6 Å, a = b = g = 90°	2.5 Å
sFcγRIII: hFcI	6% PEG 8000, 0.1M MES/TRIS pH 5.6, 0.2M Na/K tartrate, 0.02% sodium azide	P6522, a = b = 115.0 Å, c = 303.3 Å, a = b = 90°, g = 120°	3.3 Å
sFcγRIII	22% PEG 8000, 0.1M MES/TRIS pH 7.8, 0.02% sodium azide	P22121, a = 36.7 Å, b = 60.3 Å, c = 85.6 Å, a = b = g = 90°	2.5 Å

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 SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 18

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<211> LENGTH: 269

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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Glu Thr Val Thr Leu His Cys Glu Val Leu His Leu Pro Gly Ser Ser
      20             25             30

Ser Thr Gln Trp Phe Leu Asn Gly Thr Ala Thr Gln Thr Ser Thr Pro
      35             40             45

Ser Tyr Arg Ile Thr Ser Ala Ser Val Asn Asp Ser Gly Glu Tyr Arg
      50             55             60

Cys Gln Arg Gly Leu Ser Gly Arg Ser Asp Pro Ile Gln Leu Glu Ile
      65             70             75             80

His Arg Gly Trp Leu Leu Gln Val Ser Ser Arg Val Phe Thr Glu
      85             90             95

Gly Glu Pro Leu Ala Leu Arg Cys His Ala Trp Lys Asp Lys Leu Val
      100            105            110

Tyr Asn Val Leu Tyr Tyr Arg Asn Gly Lys Ala Phe Lys Phe Phe His
      115            120            125

Trp Asn Ser Asn Leu Thr Ile Leu Lys Thr Asn Ile Ser His Asn Gly
      130            135            140

Thr Tyr His Cys Ser Gly Met Gly Lys His Arg Tyr Thr Ser Ala Gly
      145            150            155            160

Ile Ser Val Thr Val Lys Glu Leu Phe Pro Ala Pro Val Leu Asn Ala
      165            170            175

Ser Val Thr Ser Pro Leu Leu Glu Gly Asn Leu Val Thr Leu Ser Cys
      180            185            190

Glu Thr Lys Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu Tyr Phe Ser
      195            200            205

Phe Tyr Met Gly Ser Lys Thr Leu Arg Gly Arg Asn Thr Ser Ser Glu
      210            215            220

Tyr Gln Ile Leu Thr Ala Arg Arg Glu Asp Ser Gly Leu Tyr Trp Cys
      225            230            235            240

Glu Ala Ala Thr Glu Asp Gly Asn Val Leu Lys Arg Ser Pro Glu Leu
      245            250            255

Glu Leu Gln Val Leu Gly Leu Gln Leu Pro Thr Pro Val
      260            265

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<210> SEQ ID NO 2

<211> LENGTH: 174

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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Met Ala Ala Pro Pro Lys Ala Val Leu Lys Leu Glu Pro Pro Trp Ile
 1             5             10             15

Asn Val Leu Gln Glu Asp Ser Val Thr Leu Thr Cys Gln Gly Ala Arg
      20             25             30

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Ser  Pro  Glu  Ser  Asp  Ser  Ile  Gln  Trp  Phe  His  Asn  Gly  Asn  Leu  Ile
   35                                40                                45

Pro  Thr  His  Thr  Gln  Pro  Ser  Tyr  Arg  Phe  Lys  Ala  Asn  Asn  Asn  Asp
   50                                55                                60

Ser  Gly  Glu  Tyr  Thr  Cys  Gln  Thr  Gly  Gln  Thr  Ser  Leu  Ser  Asp  Pro
   65                                70                                75                                80

Val  His  Leu  Thr  Val  Leu  Ser  Glu  Trp  Leu  Val  Leu  Gln  Thr  Pro  His
                        85                                90                                95

Leu  Glu  Phe  Gln  Glu  Gly  Glu  Thr  Ile  Met  Leu  Arg  Cys  His  Ser  Trp
                        100                                105                                110

Lys  Asp  Lys  Pro  Leu  Val  Lys  Val  Thr  Phe  Phe  Gln  Asn  Gly  Lys  Ser
                        115                                120                                125

Gln  Lys  Phe  Ser  Arg  Leu  Asp  Pro  Thr  Phe  Ser  Ile  Pro  Gln  Ala  Asn
                        130                                135                                140

His  Ser  His  Ser  Gly  Asp  Tyr  His  Cys  Thr  Gly  Asn  Ile  Gly  Tyr  Thr
   145                                150                                155                                160

Leu  Phe  Ser  Ser  Lys  Pro  Val  Thr  Ile  Thr  Val  Gln  Val  Pro
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<210> SEQ ID NO 3
<211> LENGTH: 185
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 3

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Met  Gly  Thr  Pro  Ala  Ala  Pro  Pro  Lys  Ala  Val  Leu  Lys  Leu  Glu  Pro
   1                                5                                10                                15

Gln  Trp  Ile  Asn  Val  Leu  Gln  Glu  Asp  Ser  Val  Thr  Leu  Thr  Cys  Arg
                        20                                25                                30

Gly  Thr  His  Ser  Pro  Glu  Ser  Asp  Ser  Ile  Gln  Trp  Phe  His  Asn  Gly
   35                                40                                45

Asn  Leu  Ile  Pro  Thr  His  Thr  Gln  Pro  Ser  Tyr  Arg  Phe  Lys  Ala  Asn
   50                                55                                60

Asn  Asn  Asp  Ser  Gly  Glu  Tyr  Thr  Cys  Gln  Thr  Gly  Gln  Thr  Ser  Leu
   65                                70                                75                                80

Ser  Asp  Pro  Val  His  Leu  Thr  Val  Leu  Ser  Glu  Trp  Leu  Val  Leu  Gln
                        85                                90                                95

Thr  Pro  His  Leu  Glu  Phe  Gln  Glu  Gly  Glu  Thr  Ile  Val  Leu  Arg  Cys
                        100                                105                                110

His  Ser  Trp  Lys  Asp  Lys  Pro  Leu  Val  Lys  Val  Thr  Phe  Phe  Gln  Asn
                        115                                120                                125

Gly  Lys  Ser  Lys  Lys  Phe  Ser  Arg  Ser  Asp  Pro  Asn  Phe  Ser  Ile  Pro
                        130                                135                                140

Gln  Ala  Asn  His  Ser  His  Ser  Gly  Asp  Tyr  His  Cys  Thr  Gly  Asn  Ile
   145                                150                                155                                160

Gly  Tyr  Thr  Leu  Tyr  Ser  Ser  Lys  Pro  Val  Thr  Ile  Thr  Val  Gln  Ala
                        165                                170                                175

Pro  Ser  Ser  Ser  Pro  Met  Gly  Ile  Ile
                        180                                185

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<211> LENGTH: 176
<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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Trp Tyr Ser Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln Gly
          20           25           30
Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu Ser
          35           40           45
Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr Val
          50           55           60
Asn Asp Ser Gly Glu Tyr Arg Cys Gln Thr Asn Leu Ser Thr Leu Ser
          65           70           75           80
Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Leu Gln Ala
          85           90           95
Pro Arg Trp Val Phe Lys Glu Glu Asp Pro Ile His Leu Arg Cys His
          100          105          110
Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn Gly
          115          120          125
Lys Asp Arg Lys Tyr Phe His His Asn Ser Asp Phe His Ile Pro Lys
          130          135          140
Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Val Gly
          145          150          155          160
Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln Gly
          165          170          175

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<210> SEQ ID NO 5

<211> LENGTH: 183

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

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Arg Ile Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn
          20           25           30
Phe Phe Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser
          35           40           45
Glu Glu Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp
          50           55           60
Ser Gly Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro
          65           70           75           80
Val Tyr Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala
          85           90           95
Glu Val Val Met Glu Gly Gln Pro Leu Phe Leu Arg Cys His Gly Trp
          100          105          110
Arg Asn Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala
          115          120          125
Leu Lys Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr
          130          135          140
Val Glu Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu
          145          150          155          160
Asp Tyr Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala Pro Arg

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165	170	175
Glu Lys Tyr Trp Leu Gln Phe		
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20 25 30		
Met Thr Gln Lys Ser Gln Ser Thr Gln Ile Ser Gln Glu Leu Glu Glu		
35 40 45		
Leu Arg Ala Glu Gln Gln Arg Leu Lys Ser Gln Asp Leu Glu Leu Ser		
50 55 60		
Trp Asn Leu Asn Gly Leu Gln Ala Asp Leu Ser Ser Phe Lys Ser Gln		
65 70 75 80		
Glu Leu Asn Glu Arg Asn Glu Ala Ser Asp Leu Leu Glu Arg Leu Arg		
85 90 95		
Glu Glu Val Thr Lys Leu Arg Met Glu Leu Gln Val Ser Ser Gly Phe		
100 105 110		
Val Cys Asn Thr Cys Pro Glu Lys Trp Ile Asn Phe Gln Arg Lys Cys		
115 120 125		
Tyr Tyr Phe Gly Lys Gly Thr Lys Gln Trp Val His Ala Arg Tyr Ala		
130 135 140		
Cys Asp Asp Met Glu Gly Gln Leu Val Ser Ile His Ser Pro Glu Glu		
145 150 155 160		
Gln Asp Phe Leu Thr Lys His Ala Ser His Thr Gly Ser Trp Ile Gly		
165 170 175		
Leu Arg Asn Leu Asp Leu Lys Gly Glu Phe Ile Trp Val Asp Gly Ser		
180 185 190		
His Val Asp Tyr Ser Asn Trp Ala Pro Gly Glu Pro Thr Ser Arg Ser		
195 200 205		
Gln Gly Glu Asp Cys Val Met Met Arg Gly Ser Gly Arg Trp Asn Asp		
210 215 220		
Ala Phe Cys Asp Arg Lys Leu Gly Ala Trp Val Cys Asp Arg Leu Ala		
225 230 235 240		
Thr Cys Thr Pro Pro Ala Ser Glu Gly Ser Ala Glu Ser Met Gly Pro		
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Leu His Ser		
275		

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 <212> TYPE: DNA
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agtgggtgaat acaggtgcca gagaggtctc tcagggcgaa gtgaccccat acagctggaa 240
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tccccactcc tggaggggaa tctggtcacc ctgagctgtg aaacaaagt gctcttcgag 600
aggcctggtt tgcagcttta cttctccttc tacatgggca gcaagacct gcgaggcagg 660
aacacatcct ctgaatacca aatactaact gctagaagag aagactctgg gttatactgg 720
tgcgaggctg ccacagagga tggaaatgtc cttaagcgca gccctgagtt ggagcttcaa 780
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<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 8

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cagtgggttc acaatgggaa tctcattccc acccacacgc agcccagcta caggttcaag 180
gccaacaaca atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgac 240
cctgtgcatc tgactgtgct ttccgaatgg ctgggtgctc agacccctca cctggagttc 300
caggagggag aaaccatcat gctgaggtgc cacagctgga aggacaagcc tctggtcaag 360
gtcacattct tccagaatgg aaaatcccag aaattctccc gtttggatcc caccttctcc 420
atcccacaag caaaccacag tcacagtggg gattaccact gcacaggaaa cataggctac 480
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<212> TYPE: DNA
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<400> SEQUENCE: 9

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ggttcaaggc caacaacaat gacagcgggg agtacacgtg ccagactggc cagaccagcc 240
tcagcgacct tgtgcatctg actgtgcttt ctgagtggct ggtgctccag acccctcacc 300
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tggtaagggt cacattcttc cagaatggaa aatccaagaa attttcccg tcggatccca 420
acttctccat cccacaagca aaccacagtc acagtgggtg ttaccactgc acaggaaaca 480

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 <213> ORGANISM: Homo sapiens

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tccacacagt ggtttcacia tgagagcctc atctcaagcc aggcctcgag ctacttcatt	180
gacgctgcca cagtcaacga cagtggagag tacagtgcc agacaaacct ctccaccctc	240
agtgaccggg tgcagctaga agtccatata ggctggctgt tgctccaggc ccctcgggtg	300
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cataaggta catatttaca gaatggcaaa gacaggaagt atttcatca taattctgac	420
ttccacattc caaaagccac actcaaagat agcggctcct acttctgcag ggggcttgtt	480
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 <211> LENGTH: 560
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

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aaatggttcc acaatggcag cctttcagaa gagacaaatt caagtttgaa tattgtgaat	180
gccaaatttg aagacagtgg agaatacaaa tgtcagcacc aacaagttaa tgagagtga	240
cctgtgtacc tggaagtctt cagtgactgg ctgctccttc aggcctctgc tgaggtggtg	300
atggagggcc agcccctctt cctcaggctc catggttgga ggaactggga tgtgtacaag	360
gtgatctatt ataaggatgg tgaagctctc aagtactggt atgagaacca caacatctcc	420
attacaaatg ccacagtta agacagtga acctactact gtacgggcaa agtgtggcag	480
ctggactatg agtctgagcc cctcaacatt actgtaataa aagctccgcg tgagaagtac	540
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 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

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tatgcctgtg acgacatgga agggcagctg gtcagcatcc acagcccga ggagcaggac	180
ttcctgacca agcatgccag ccacaccggc tcctggattg gccttcggaa cttggacctg	240
aagggggagt ttatctgggt ggatgggagc cagctggact acagcaactg ggtccaggg	300

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acgccgccag ccagcgaagg ttccgcggag tccatgggac ctgattcaag accagaccct 480
gacgggccgc tgccccccc ctctgcccc ctccactctt gagcatggat cc 532

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<211> LENGTH: 1419
<212> TYPE: DNA
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<400> SEQUENCE: 13

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gtcatatgct tctgtggaca gctgtgctat tcctggctcc tgttgctggg acacctgcag 180
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<210> SEQ ID NO 14
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 14

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<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

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ccc	1503

1-40. (canceled)

41. A recombinant soluble Fc receptor having no transmembrane domains and no signal peptide, and where no glycosylation occurs.

42. The recombinant soluble Fc receptor according to claim 41, wherein the receptor is a FcγR or a FcεR.

43. The recombinant soluble Fc receptor according to claim 41, wherein the receptor is FcγRIIb.

44. The recombinant soluble Fc receptor according to claim 41, wherein the receptor is of human origin.

45. The recombinant soluble Fc receptor according to claim 41, wherein the receptor contains one of the amino acids as shown in one of SEQ ID Nos: 1-6

46. The recombinant nucleic acid containing a sequence encoding a recombinant Fc receptor according to claim 41, wherein said nucleic acid is contained on a prokaryotic expression vector.

47. The recombinant nucleic acid according to claim 46, wherein said nucleic acid contains one of the sequences shown in one of SEQ ID Nos: 7-12

48. The recombinant nucleic acid according to claim 46, wherein said nucleic acid additionally contains expression control sequences operably linked to the sequence encoding the recombinant Fc receptor.

49. A host cell characterized by the presence of a recombinant nucleic acid according to claim 46, wherein said cell is a prokaryotic host cell.

50. A process for the determination of the amount of antibodies of a certain Ig class in the blood, plasma or serum of a patient, comprising the use of a recombinant soluble Fc receptor according to claim 41 in an immunoassay and the determination of the presence of FcR-antibody complexes.

51. The process according to claim 50, wherein the immunoassay is an ELISA assay.

52. The process according to claim 50, wherein the antibodies to be determined are IgE antibodies and the recombinant soluble receptor is a FcεR.

53. The process according to claim 52 for determination of a predisposition of manifestation of an allergy.

54. The process according to claim 50, wherein the antibodies to be determined are IgG antibodies and the recombinant soluble receptor is a FcγR.

55. A process for determination of the immune status of patients with chronic diseases of the immune system, wherein a Fc receptor according to claim 41 is used in a competitive immunoassay and the amount of the corresponding sFcRs in the blood, plasma or serum of a patient is determined.

56. The process according to claim 55, wherein the chronic disease is AIDS, SLE, MM or rheumatoid arthritis.

57. The use of a recombinant soluble Fc receptor according to claim 41 for the screening of substances for their ability to act as inhibitors of the recognition and binding of antibodies to cellular receptors.

58. The use of a recombinant soluble Fc receptor according to claim 57, wherein recombinant soluble FcγRs are used and recognition and binding of IgG antibodies is of interest.

59. A pharmaceutical composition containing as active agent a recombinant soluble FcR according to claim 41.

60. The pharmaceutical composition of claim 59 for use in the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

61. The pharmaceutical composition of claim 59 for use in treatment of AIDS, rheumatoid arthritis or multiple myeloma, containing a recombinant soluble FcγR preferably having the amino acid sequence as shown in SEQ ID No.:1

62. A crystalline preparation of a soluble recombinant Fc receptor according to claim 41.

63. A crystalline preparation of a soluble recombinant Fc receptor/immunoglobulin complex.

64. The use of a crystalline preparation of a soluble recombinant Fc receptor according to claim 41 for generation of crystal structure data of Fc receptors.

65. The use of a crystalline preparation of a soluble recombinant Fc receptor/immunoglobulin complex for generation of crystal structure data of receptor/Ig complexes and their respective binding sites.

66. The use of crystal structure data obtained by use according to claim 64 for identification and/or preparation of Fc receptor or immunoglobulin inhibitors.

67. The use of crystal structure data, obtained by use according to claim 65, for identification and preparation of new antibody receptors.

68. The use of a crystalline preparation of a soluble recombinant Fc receptor according to claim 64 in a computer-aided modeling program.

69. An FcR inhibitor having a three-dimensional structure which is complementary to the recombinant soluble FcR of claim 41.

70. An immunoglobulin-inhibitor, having a three-dimensional structure which is complementary to an Fc receptor binding site of an immunoglobulin.

71. A pharmaceutical composition containing a FcR inhibitor according to claim 69.

72. A pharmaceutical composition containing an immunoglobulin inhibitor according to claim 70.

73. The pharmaceutical composition according to claim 71 for use in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system.

74. The pharmaceutical composition according to claim 71 for treatment or prevention of allergies, autoimmune diseases or an anaphylactic shock.

75. The use of a molecule for modulation of the interaction between an Fc receptor and immunoglobulin, wherein the molecule is designed or identified using crystal structure data obtained from crystalline preparations according to claim 62.

76. The use of a molecule for modulation of the interaction between an Fc receptor and immunoglobulin according to claim 75, wherein the modulation is partial or complete inhibition of binding between Fc receptor and immunoglobulin.

77. The Fc receptor of claim 41, bound to a solid phase.

78. The Fc receptor of claim 77, wherein the solid phase is a chromatography carrier material.

79. The use of a chromatography carrier material according to claim 78 for adsorption of immunoglobulins from the blood, plasma or serum of a patient or from culture supernatants of immunoglobulin producing cells.

80. The use of a chromatography carrier material according to claim 79, for enrichment of antibodies from a patient's blood, serum or plasma or from culture supernatants of immunoglobulin producing cells for conduction of further tests.

* * * * *

专利名称(译)	重组可溶性Fc受体		
公开(公告)号	US20070207163A1	公开(公告)日	2007-09-06
申请号	US11/327695	申请日	2006-01-06
[标]申请(专利权)人(译)	SONDERMANN PETER ROBERT RUBER JACOB UWE		
申请(专利权)人(译)	SONDERMANN PETER ROBERT RUBER JACOB UWE		
当前申请(专利权)人(译)	SONDERMANN PETER ROBERT RUBER JACOB UWE		
[标]发明人	SONDERMANN PETER RUBER ROBERT JACOB UWE		
发明人	SONDERMANN, PETER RUBER, ROBERT JACOB, UWE		
IPC分类号	A61K39/00 A61K48/00 C07H21/04 C07K16/00 G01N33/53 A61K38/00 A61K38/17 A61K45/00 A61P19/02 A61P29/00 A61P31/18 A61P35/00 A61P37/02 A61P37/08 C07K14/705 C07K14/735 C07K17/00 C12N1/21 C12N15/09 C12N15/12 C12N15/70 C12R1/19 G01N33/68		
CPC分类号	A61K38/00 C07K2299/00 C07K14/70535 A61P19/02 A61P29/00		
优先权	1998122969 1998-12-03 EP 09/856933 2002-02-27 US PCT/EP1999/009440 1999-12-03 WO		
其他公开文献	US7504482		
外部链接	Espacenet USPTO		

摘要(译)

根据本发明的重组可溶性Fc受体的特征在于不存在跨膜结构域，信号肽和糖基化。通过在原核宿主细胞中表达各自的核酸和获得的包涵体的复性，可以容易地获得这样的Fc受体，该过程产生非常均匀和纯的产物。该产品可用于诊断和制药应用，也可用于生成晶体结构数据。这种晶体结构数据可用于人工分子的建模。另一个实施方案包括将根据本发明的Fc受体偶联至固体材料，如可用于分离和/或富集抗体的色谱材料。

