20 (2009) 592-599). In WO 01/03737 immunoglobulin fusion proteins are reported. The expression and export of anti-obesity proteins as Fc fusion proteins is reported in WO 00/40615.

Summary of the Invention

It has been found that soluble Fc-receptors can be produced by expressing the Fc-receptor as fusion polypeptide with an Fc-region that does not substantially bind to the fused Fc-receptor. Using a fusion polypeptide for the expression of the Fc-receptor increases the obtainable yield of the Fc-receptor either in form of the fusion polypeptide or as isolated receptor. Additionally the fusion polypeptide as reported herein provides for an increased flexibility for combining more than one copy of an Fc-receptor in a single molecule, e.g. for increased avidity, or for combining different Fc-receptors (of different origin or of different type or both) in a single molecule.

There is ~~One aspect as~~ reported herein ~~is~~ a fusion polypeptide according to formula I

R1-FC-R2 (formula I)

wherein
R1 denotes a first Fc-receptor,
R2 denotes a second Fc-receptor, and
FC denotes a heavy chain Fc-region polypeptide,
wherein R1 or R2 or both are present,
wherein FC does not substantially bind to R1 and/or R2.

One aspect as reported herein is a fusion polypeptide according to formula I

R1-FC-R2 (formula I)

wherein
R1 denotes a first Fc-receptor,
R2 denotes a second Fc-receptor, and
FC denotes a heavy chain Fc-region polypeptide,
wherein R1 or R2 or both are present,
wherein FC does not substantially bind to R1 and/or R2,
wherein R1 and R2 are independently of each other selected from the group consisting of human Fcgamma-receptor, human neonatal Fc-receptor, murine Fc-receptor, and rabbit neonatal Fc-receptor, and

wherein FC is human IgG1 heavy chain polypeptide with the mutations L234A, L235A and P329G.

In one embodiment the fusion polypeptide has the formula II

R1-CS1-L1-CS2-FC-CS3-L2-CS4-R2 (formula II)

wherein
R1 denotes a first Fc-receptor,
R2 denotes a second Fc-receptor,
FC denotes a heavy chain Fc-region polypeptide,
CS1 denotes a first cleavage site,
CS2 denotes a second cleavage site,
CS3 denotes a third cleavage site,
CS4 denotes a fourth cleavage site,
L1 denotes a first intervening amino acid sequence, and

f) R2 of the first and second fusion polypeptide are identical and both R1 are absent,

g) R2 of the first and second fusion polypeptide are different and both R1 are absent,

h) R1 of the first fusion polypeptide and R2 of the second polypeptide are different and R2 of the first fusion polypeptide is absent and R1 of the second polypeptide is absent.

There is ~~One aspect as~~ reported herein ~~is~~ a method for the production of a soluble Fc-receptor comprising the following steps

a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide as reported herein,

b) recovering the fusion polypeptide from the cell or the cultivation medium,

c) optionally cleaving the fusion polypeptide with a protease,

thereby producing a soluble Fc-receptor.

One aspect as reported herein is a method for the production of a soluble Fc-receptor comprising the following steps

a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide as reported herein,

b) recovering the fusion polypeptide from the cell or the cultivation medium,

and thereby producing a soluble Fc-receptor

One aspect as reported herein is the use of an immobilized fusion polypeptide or an immobilized dimeric fusion polypeptide as reported herein as affinity chromatography ligand.

One aspect as reported herein is the use of an immobilized fusion polypeptide or an immobilized dimeric fusion polypeptide as reported herein for determining the Fc-receptor binding of an antibody.

In one embodiment the fusion polypeptide is bound to a solid phase.

One aspect as reported herein is a pharmaceutical composition comprising a fusion polypeptide as reported herein.

One aspect as reported herein is the use of a fusion polypeptide as reported herein in the manufacture of a medicament.

In one embodiment the medicament is for the treatment of an inflammatory disease.

In one embodiment the disease is a disease characterized by increased antibody levels.

In one embodiment the disease is an autoimmune disease.

Patent Claims

1. A fusion polypeptide according to formula I

R1-FC-R2 (formula I)

wherein
R1 denotes a first Fc-receptor,
R2 denotes a second Fc-receptor, and
FC denotes a heavy chain Fc-region polypeptide,
wherein R1 or R2 or both are present,
wherein FC does not substantially bind to R1 and/or R2,

wherein R1 and R2 are independently of each other selected from the group consisting of human Fcgamma-receptor, human neonatal Fc-receptor, murine Fc-receptor, and rabbit neonatal Fc-receptor, and

wherein FC is human IgG1 heavy chain polypeptide with the mutations L234A, L235A and P329G.

2. The fusion polypeptide according to claim 1, characterized in that the fusion polypeptide has the formula II

R1-CS1-L1-CS2-FC-CS3-L2-CS4-R2 (formula II)

wherein
R1 denotes a first Fc-receptor,
R2 denotes a second Fc-receptor,
FC denotes a heavy chain Fc-region polypeptide,
CS1 denotes a first cleavage site,
CS2 denotes a second cleavage site,
CS3 denotes a third cleavage site,
CS4 denotes a fourth cleavage site,
L1 denotes a first linker, and
L2 denotes a second linker,
wherein R1 or R2 or both are present,
wherein any one of CS1, CS2, CS3, CS4 can independently of each other be present or absent,
wherein L1 and L2 can independently of each other be present or absent,
wherein FC does not substantially bind to R1 and/or R2,

wherein R1 and R2 are independently of each other selected from the group consisting of human Fcgamma-receptor, human neonatal Fc-receptor, murine Fc-receptor, and rabbit neonatal Fc-receptor, and

wherein FC is human IgG1 heavy chain polypeptide with the mutations L234A, L235A and P329G.

3. The fusion polypeptide according to any one of claims 1 or 2, characterized in that the human Fcgamma-receptor is selected from human FcγRI, human FcγRII, human FcγRIIA, human FcγRIIB, human FcγRIIC, human FcγRIII, human FcγRIIIA, and human FcγRIIIB.

4. The fusion polypeptide according to any one of claims 1 to 3, characterized in that the human Fcgamma-receptor is selected from human FcγRIA, human FcγRIB, human FcγRIC, human FcγRIIA, human FcγRIIA allotype H131, human FcγRIIA allotype R131, human FcγRIIB, human FcγRIIB-1, human FcγRIIB-2, human FcγRIIc, human FcγRIIIA, human FcγRIIIA allotype, human FcγRIIIA allotype V158, human FcγRIIIA allotype F158, human FcγRIIIb, human FcγRIIIb allotype FcγRIIB-NA1, human FcγRIIIb allotype FcγRIIB-NA2, human FcγR isoforms.

5. The fusion polypeptide according to any one of claims 3 or 4, characterized in that the human Fcgamma-receptor is FcgammaRIIIaV158.

~~3. The fusion polypeptide according to any one of the preceding claims, characterized in that R1 and R2 are independently of each other selected from the group of human Fcgamma-receptor, human neonatal Fc-receptor, murine Fc-receptor, and rabbit neonatal Fc-receptor.~~

~~4. The fusion polypeptide according to any one of the preceding claims, characterized in that FC is a variant of a heavy chain polypeptide selected from the group of human IgG1 heavy chain polypeptide, human IgG2 heavy chain polypeptide, human IgG3 heavy chain polypeptide, human IgG4 heavy chain polypeptide, murine IgG1 heavy chain polypeptide, murine IgG2 heavy chain polypeptide, murine IgG2a heavy chain polypeptide, murine IgG3 heavy chain polypeptide, and rabbit IgG heavy chain polypeptide.~~

~~5. The fusion polypeptide according to any one of the preceding claims, characterized in that FC is selected from human IgG1 heavy chain polypeptide with the mutations L234A, L235A and P329G, human IgG4 heavy chain polypeptide with the mutation S228P and L235E, human IgG1 heavy chain polypeptide with the mutation I253A, H310A and A435A.~~

6. A dimeric fusion polypeptide comprising two fusion polypeptides according to any one of claims 1 to 5.

7. The fusion polypeptide according to claim 6, characterized in that the first FC comprises the mutation T366W and optionally the mutation S354C and the second FC comprises the mutations T366S, L368A and Y407V and optionally the mutation Y349C.

8. The fusion polypeptide according to any one of claims 6 to 7, characterized in that

a) R1 and R2 of the first and second polypeptide are identical,

b) R1 and R2 of the first fusion polypeptide are identical, R1 and R2 of the second fusion polypeptide are identical but different from R1 and R2 of the first fusion polypeptide,

c) R1 of the first and second fusion polypeptide are identical and R2 of the first and second polypeptide are identical but different from R1,

d) R1 of the first and second fusion polypeptide are identical and both R2 are absent,

e) R1 of the first and second fusion polypeptide are different and both R2 are absent,

f) R2 of the first and second fusion polypeptide are identical and both R1 are absent,

g) R2 of the first and second fusion polypeptide are different and both R1 are absent,

h) R1 of the first fusion polypeptide and R2 of the second polypeptide are different and R2 of the first fusion polypeptide is absent and R1 of the second polypeptide is absent.

9. Method for the production of a soluble Fc-receptor comprising the following steps
a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide according to any one of claims 1 to 6,
b) recovering the fusion polypeptide from the cell or the cultivation medium,
~~c) optionally cleaving the fusion polypeptide with a protease,~~
and thereby producing a soluble Fc-receptor.

10. Method according to claim 9, further comptising the step of cleaving the fusion polypeptide with a protease.

~~10~~11. Use of an immobilized fusion polypeptide according to any one of claims 1 to 8 as affinity chromatography ligand.

~~11~~12. Use of an immobilized fusion polypeptide according to any one of claims 1 to 8 for determining the Fc-receptor binding of an antibody.

~~12~~13. The use according to any one of claims ~~10~~ 11 to ~~11~~12, characterized in that the fusion polypeptide is bound to a solid phase.

~~13~~14. Pharmaceutical composition comprising a fusion polypeptide according to any one of claims 1 to 8.

~~14~~15. Use of a fusion polypeptide according to any one of claims 1 to 8 in the manufacture of a medicament.

~~15~~16. The use according to claim ~~14~~15, characterized in that the medicament is for the treatment of an inflammatory disease.