According to a first aspect, there is provided a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a. A first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain,
b. A second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,
c. A third nucleic acid molecule encoding a 3rd CH3 domain-comprising polypeptide chain, and
d. A fourth nucleic acid molecule encoding a 4th CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid 351~~at least two of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3-domain comprising polypeptides~~, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acid molecules and harvesting said at least two different Ig-like molecules from the culture.

According to a second aspect, there is provided a mixture of at least two different Ig-like molecules obtainable by the method as defined herein.

According to a third aspect, there is provided a recombinant host cell comprising nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3-domain-comprising polypeptide chain, wherein ~~at least two of said nucleic acid sequences are provided with means for preferential pairing of~~ said first ~~and second~~ CH3-domain-comprising polypeptide~~s~~ chain comprises a lysine (K) residue at amino acid position 366 and wherein said second ~~and said third and fourth~~ CH3-domain-comprising polypeptide~~s~~ chain comprises an aspartic acid (D) residue at amino acid 351.

According to a fourth aspect, there is provided a pharmaceutical composition comprising the at least two different Ig-like molecules as defined herein, and a pharmaceutically acceptable carrier.

According to a fifth aspect, there is provided a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3-domain-comprising polypeptide chain, wherein ~~at least two of said nucleic acid sequences are provided with means for preferential pairing of~~ said first ~~and second~~ CH3-domain-comprising polypeptides chain comprises a lysine (K) residue at amino acid position 366 and wherein said second ~~and said third and fourth~~ CH3-domain-comprising polypeptides chain comprises an aspartic acid (D) residue at amino acid 351, and wherein said nucleic acid sequences are introduced consecutively or concomitantly.

According to a sixth aspect, there is provided a culture of recombinant host cells as defined herein, or of recombinant host cells obtained by the method as defined herein, producing at least two different Ig-like molecules.

Claims

1. A method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a. A first nucleic acid molecule encoding a 1st CH3 domain-comprising polypeptide chain,

b. A second nucleic acid molecule encoding a 2nd CH3 domain-comprising polypeptide chain,

c. A third nucleic acid molecule encoding a 3rd CH3 domain-comprising polypeptide chain, and

d. A fourth nucleic acid molecule encoding a 4th CH3 domain-comprising polypeptide chain,

wherein ~~at least two of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3-domain-comprising polypeptides~~ said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid 351, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acid molecules and harvesting said at least two different Ig-like molecules from the culture.

2. The method of claim 1, further comprising providing said host cell with a nucleic acid molecule encoding a common light chain.

~~3. The method of claim 1 or 2, wherein said first CH3-domain-comprising polypeptide chain comprises the amino acid substitution T366K, and said second CH3-domain comprising polypeptide chain comprises the amino acid substitution L351D.~~

43. The method of claim ~~3~~1, wherein said first CH3_-domain_-comprising polypeptide chain further comprises ~~the amino acid substitution L351K~~ a lysine (K) residue at amino acid position 351.

~~54~~. The method of claim 1 or ~~3 or 4~~, wherein said second CH3_-domain_-comprising polypeptide chain further comprises ~~an amino acid substitution selected from the group consisting of Y349E, Y349D and L368E~~ a glutamic acid (E) residue or an aspartic acid (D) residue at amino acid position 349, and/or a glutamic acid (E) residue at amino acid position 368.

~~65~~. The method of claim ~~54~~, wherein said second CH3_-domain_-comprising polypeptide chain further comprises ~~the amino acid substitution L368E~~ a glutamic acid (E) residue at amino acid position 368.

~~76~~. The method of any one of claims 1-~~65~~, wherein said third CH3_-domain_-comprising polypeptide chain comprises ~~the amino acid substitutions E356K and D399K~~ a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 399, and said fourth CH3-domain ~~comprising polypeptide chain comprises the amino acid substitutions K392D and K409D~~ comprises an aspartic acid (D) residue at amino acid position 392 and an aspartic acid (D) residue at amino acid position 409.

~~87~~. The method of any one of claims 1-~~76~~, wherein each of the CH3_-domain_-comprising polypeptide chains further comprises a variable region recognizing a target epitope.

~~98~~. The method of claim ~~87~~, wherein each of the 4 variable regions of the 4 CH3_-domain_-comprising polypeptide chains recognizes different target epitopes.

~~109~~. The method of claim ~~87~~, wherein the variable regions of the 1st and the 2nd CH3_-domain_-comprising polypeptide chains recognize different target epitopes, whereas the variable regions of the 3rd and the 4th CH3_-domain_-comprising polypeptide chains recognize the same target epitopes.

~~1110~~. The method of claim ~~10 9~~ wherein the target epitope recognized by the variable regions of the 3rd and 4th CH3 domain_-comprising polypeptide chain is the same as the target epitope recognized by the variable region of the 1st or the 2nd CH3_-domain_-comprising polypeptide chain.

~~12~~11. The method of claim ~~10~~ 9 wherein the target epitope recognized by the variable regions of the 3rd and 4th CH3 domain-comprising polypeptide chain is different from the target epitope recognized by the variable region of the 1st or the 2nd CH3-domain-comprising polypeptide chain.

~~13~~12. The method of claim ~~8~~7, wherein the variable regions of the 1st and the 2nd CH3-domain-comprising polypeptide chains recognize the same target epitope, whereas the variable regions of the 3rd and the 4th CH3-domain-comprising polypeptide chains recognize a second target epitope which differs from the target epitope recognized by said 1st and 2nd variable regions.

~~14~~13. The method of any one of claims ~~8~~7-~~13~~12 wherein the target epitopes are located on the same target molecule.

~~15~~14. The method of claim ~~14~~13 wherein the target molecule is a soluble molecule.

~~16~~15. The method of claim ~~14~~13 wherein the target molecule is a membrane-bound molecule.

~~17~~16. The method of any one of claims ~~8~~7-~~13~~12 wherein the target epitopes are located on different target molecules.

~~18~~17. The method of claim ~~17~~16, wherein the different target molecules are expressed on the same cells.

~~19~~18. The method of claim ~~17~~16, wherein the different target molecules are expressed on different cells.

~~20~~19. The method of claim ~~17~~16, wherein the different target molecules are soluble molecules.

~~21~~20. The method of claim ~~17~~16, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

~~22~~21. The method according to any one of claims 1-~~21~~20, wherein said at least two different Ig-like molecules are antibodies.

~~23~~22. The method of claim 1, wherein ~~said means for preferential pairing comprises~~at least two of said nucleic acid molecules are provided with engineered complementary knob-into-hole mutations, disulfide bridges, charge mutations or combinations thereof.

~~24~~23. The method of claim ~~23~~22, wherein said ~~means for preferential pairing~~charge mutations are selected from Table B.

~~25. The method of claim 1, wherein all 4 of said nucleic acid molecules are provided with means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides and said 3rd and 4th CH3 domain-comprising polypeptides, wherein said means for preferential pairing of said 1st and 2nd CH3 domain-comprising polypeptides are different from those means for preferential pairing of said 3rd and 4th CH3 domain-comprising polypeptides.~~

~~26~~24. The method of any one of claims ~~8~~7-~~13~~12, ~~17~~16-~~19~~18 or ~~21~~20, wherein at least one of said target epitopes is located on a tumor cell.

~~27~~25. The method of any one of claims ~~8~~7-~~13~~12, ~~17~~16-~~19~~18 or ~~21~~20, wherein at least one of said target epitopes is located on an effector cell.

~~28~~26. The method of claim ~~27~~25, wherein said effector cell is an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

~~29~~27. The method of claims ~~27~~25 or ~~28~~26, wherein said target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

~~30~~28. A mixture of at least two different Ig-like molecules obtainable by the methods according to any one of claims 1-~~29~~27.

~~31~~29. A mixture according to claim ~~30~~28, wherein said at least two different Ig-like molecules bind to different epitopes on the same antigen and/or to different epitopes on different antigens.

~~32~~30. A mixture according to claim ~~30~~ 28 or ~~31~~29, wherein said at least two different Ig-like molecules comprises at least one heterodimeric Ig-like molecule.

~~33~~31. A mixture according to any one of claims 28 ~~30~~ ~~32~~, wherein two of said at least two different Ig-like molecules are heterodimeric Ig-like molecules.

~~34~~32. A recombinant host cell comprising nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3 -domain-comprising polypeptide chain, wherein ~~at least two of said nucleic acid sequences are provided with means for preferential pairing of said first and second~~said first CH3 -domain-comprising polypeptide~~s and said third and fourth~~ chain comprises a lysine (K) residue at amino acid position 366 and wherein said second CH3 -domain-comprising polypeptide~~s~~ chain comprises an aspartic acid (D) residue at amino acid 351.

~~35~~33. A recombinant host cell according to claim ~~34~~32, wherein said host cell further comprises a nucleic acid sequence encoding a common light chain.

~~36~~34. A pharmaceutical composition comprising the at least two different Ig-like molecules of any one of claims ~~30~~28-~~33~~31, and a pharmaceutically acceptable carrier.

~~37~~35. A pharmaceutical composition according to claim ~~36~~34, wherein said at least two different Ig-like molecules have been produced by recombinant host cells according to claims ~~34~~ 32 or ~~35~~33.

~~38~~36. A method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell nucleic acid sequences encoding at least a first, a second, a third and a fourth CH3 -domain-comprising polypeptide chain, wherein ~~at least two of said nucleic acid sequences are provided with means for preferential pairing of said first and second~~said first CH3 -domain-comprising polypeptide~~s~~ chain comprises a lysine (K) residue at amino acid position 366 and wherein said second ~~and said third and fourth~~ CH3 -domain-comprising polypeptide~~s~~ chain comprises an aspartic acid (D) residue at amino acid 351, and wherein said nucleic acid sequences are introduced consecutively or concomitantly.

~~39~~37. A method according to claim ~~38~~36, further comprising the step of introducing into said host cell a nucleic acid sequence encoding a common light chain.

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~~40~~38. A culture of recombinant host cells of claims ~~34~~-32 or ~~35~~33, or of recombinant host cells obtained by the method according to claims ~~38~~-36 or ~~39~~37, producing at least two different Ig-like molecules.