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

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

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

wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and ~~first CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue and~~ wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid 351~~at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue~~, said method further comprising the step of culturing said host cell and allowing for expression of said two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule from the culture.

According to a second aspect, there is provided a heterodimeric Ig-like molecule obtainable by the method as defined herein.

According to a third aspect, there is provided a heterodimeric antibody comprising two CH3 domains, wherein one of said two CH3 domains comprises an aspartic acid (D) residue at amino acid 351 and a glutamic acid (E) residue at amino acid position 368 ~~the amino acid substitutions L351D and L368E~~ and wherein the other of said two CH3 domains comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351~~the amino acid substitutions T366K and L351K~~.

According to a fourth aspect, there is provided a recombinant host cell comprising nucleic acid sequences encoding at least a first and a second CH3-domain comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 ~~at least one substitution of a neutral amino acid residue by a positively charged amino acid residue~~ and wherein said second CH3 domain-comprising polypeptide chain comprises at an aspartic acid (D) residue at amino acid 351~~least one substitution of a neutral amino acid residue by a negatively charged amino acid residue~~.

According to a fifth aspect, there is provided a pharmaceutical composition comprising a heterodimeric Ig-like molecule as defined herein or a heterodimeric antibody as defined herein, and a pharmaceutically acceptable carrier.

According to a sixth aspect, there is provided a method for making a host cell for production of a heterodimeric Ig-like molecule, the method comprising the step of introducing into said host cell nucleic acid sequences encoding at least a first and a second 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 one substitution of a neutral amino acid residue by a positively charged amino acid residue~~ and ~~wherein said second CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue,~~ wherein said nucleic acid sequences are introduced consecutively or concomitantly.

According to a seven aspect, there is provided a culture of recombinant host cells as defined herein, or of recombinant host cells obtained by a method as defined herein, producing a heterodimeric Ig-like molecule.

Claims

1. A method for producing a heterodimeric Ig-like molecule from a single cell, wherein said Ig-like molecule comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

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

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

wherein said ~~first CH3 domain-comprising polypeptide chain comprises at least one substitution of a neutral amino acid residue by a positively charged amino acid residue and~~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 ~~at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue~~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 two nucleic acid molecules and harvesting said heterodimeric Ig-like molecule 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 wherein 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.

~~5~~4. 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 according to any one of claims 1 to ~~65~~, wherein the presence of contaminating homodimers is less than 5%, preferably less than 2%, more preferably less than 1%, and most preferably contaminating homodimers are essentially absent.

~~87~~. The method according to any one of claims 1 to ~~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 2 variable regions of the CH3_-domain_-comprising polypeptide chains recognizes a different target epitope.

~~109~~. The method of claim ~~98~~, wherein the different target epitopes are located on the same target molecule.

~~1110~~. The method of claim ~~109~~, wherein the target molecule is a soluble molecule.

~~1211~~. The method of claim ~~109~~, wherein the target molecule is a membrane-bound molecule.

~~1312~~. The method of claim ~~98~~, wherein the different target epitopes are located on different target molecules.

~~1413~~. The method of claim ~~1312~~, wherein the different target molecules are expressed on the same cells.

~~1514~~. The method of claim ~~1312~~, wherein the different target molecules are expressed on different cells.

~~1615~~. The method of claim ~~1312~~, wherein the different target molecules are soluble molecules.

~~17~~16. The method of claim ~~13~~12, wherein one target molecule is a soluble molecule whereas the second target molecule is a membrane bound molecule.

~~18~~17. The method of any one of claims ~~8~~7-~~10~~9, ~~12~~11-~~15~~14 or ~~17~~16, wherein at least one of said target epitopes is located on a tumor cell.

~~19~~18. The method of any one of claims ~~8~~7-~~10~~9, ~~12~~11-~~15~~14 or ~~17~~16, wherein at least one of said target epitopes is located on an effector cell.

~~20~~19. The method of claim ~~19~~18, 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.

~~21~~20. The method of claim 18 or 19 ~~or 20~~, wherein said target epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

~~22~~21. The method according to any one of claims 1-~~21~~20, wherein said heterodimeric Ig-like molecule is an antibody, preferably a human IgG, most preferably a human IgG1.

~~23~~22. The method according to any one of claims 2-~~22~~21, wherein said common light chain is a germline light chain, preferably the rearranged germline human kappa light chain IgVκ1-39*01/IGJκ1*01.

~~24~~23. A heterodimeric Ig-like molecule obtainable by the methods according to any one of claims 1-~~23~~22.

~~25~~24. A heterodimeric Ig-like molecule according to claim ~~24~~23, wherein said heterodimeric Ig-like molecule binds to different epitopes on the same antigen and/or to different epitopes on different antigens.

~~26~~25. A heterodimeric Ig-like molecule according to claim ~~24~~23 or ~~25~~24, wherein said Ig-like molecule is an antibody.

~~27~~26. A heterodimeric antibody comprising two CH3 domains, wherein one of said two CH3 domains comprises ~~the amino acid substitutions L351D and L368E~~ an aspartic acid (D) residue at amino acid 351 and a glutamic acid (E) residue at amino acid position 368 and

wherein the other of said two CH3 domains comprises ~~the amino acid substitutions T366K and L351K~~ a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351.

~~28~~27. A recombinant host cell comprising nucleic acid sequences encoding at least a first and a second CH3_-domain_-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises ~~at least one substitution of a neutral amino acid residue by a positively charged amino acid residue~~ a lysine (K) residue at amino acid position 366 and wherein said second CH3 domain-comprising polypeptide chain comprises ~~at least one substitution of a neutral amino acid residue by a negatively charged amino acid residue~~an aspartic acid (D) residue at amino acid 351.

~~29~~28. A recombinant host cell according to claim ~~28~~27, wherein said host cell further comprises a nucleic acid sequence encoding a common light chain.

~~30~~29. A pharmaceutical composition comprising a heterodimeric Ig-like molecule according to any one of claims ~~24~~23-~~26~~25 or a heterodimeric antibody according to claim ~~27~~26, and a pharmaceutically acceptable carrier.

~~31~~30. A pharmaceutical composition according to claim ~~30~~29, wherein said heterodimeric Ig-like molecule or heterodimeric antibody has been produced by recombinant host cells according to claims 27 or 28 ~~or 29~~.

~~32~~31. A method for making a host cell for production of a heterodimeric Ig-like molecule, the method comprising the step of introducing into said host cell nucleic acid sequences encoding at least a first and a second CH3_-domain_-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises ~~at least one substitution of a neutral amino acid residue by a positively charged amino acid residue~~ 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 one substitution of a neutral amino acid residue by a negatively charged amino acid residue~~, and wherein said nucleic acid sequences are introduced consecutively or concomitantly.

~~33~~32. A method according to claim ~~32~~31, further comprising the step of introducing into said host cell a nucleic acid sequence encoding a common light chain.

~~34~~33. A culture of recombinant host cells according to claim 27 or 28 ~~or 29~~, or of recombinant host cells obtained by a method according to claim 31 or 32 ~~or 33~~, producing a heterodimeric Ig-like molecule.