Binding Molecules

Field of the Invention

The present invention relates to improved methods for isolating from transgenic non-human mammals a diverse repertoire of functional, soluble, heavy chain-only antibodies and soluble VH domains derived therefrom which are structurally distinct from VH domains derived from antibodies comprising heavy and light chains. Preferably, the heavy chain-only antibodies and soluble VH domains are derived from long-lived plasma cells or memory B-cells.

Background to the Invention

Monoclonal antibodies or variants thereof will represent a high proportion of new medicines launched in the 21st century. Monoclonal antibody therapy is already accepted as a preferred route for the treatment for rheumatoid arthritis and Crohn's disease and there is impressive progress in the treatment of cancer. Antibody-based products are also in development for the treatment of cardiovascular and infectious diseases. Most marketed monoclonal antibody products recognise and bind a single, well-defined epitope on the target ligand (e.g. TNFα).

The structure of antibodies is well known in the art. Most natural antibodies are tetrameric and comprise two heavy chains and two light chains. The heavy chains are joined to each other via disulphide bonds between hinge domains located approximately half way along each heavy chain. A light chain is associated with each heavy chain on the N-terminal side of the hinge domain. Each light chain is normally bound to its respective heavy chain by a disulphide bond close to the hinge domain.

When an antibody molecule is correctly folded, each chain folds into a number of distinct globular domains joined by a more linear polypeptide sequence. For example, the light chain folds into a variable (VL) and a constant (CL) domain. Heavy chains have a single variable domain (VH) adjacent the variable domain (VL) of the light chain, a first constant domain (CH1), a hinge domain and two or three further constant domains. Interaction of the heavy (VH) and light (VL) chain variable domains results in the formation of an antigen-binding region (Fv).

The interaction between the heavy and light chains is mediated by the first constant domain of the heavy chain (CH1) and the constant domain (CL) of the light chain. However, there is also an interface between the heavy chain variable domain

CLAIMS

1. A high affinity, antigen-specific, soluble heavy chain-only antibody which:

lacks hallmark camelid-related amino acid substitutions and has FR2 substitutions which are not found in antibodies which comprise heavy and light chain;

shows increased net hydrophobicity within CDR1 and an increased number of charged amino acids present in CDR3; and

comprises one or more amino acid substitutions within the framework ß-pleated sheet leading to increased net hydrophobicity within FR1 and an increased number of charged amino acids present in FR3.

2. The heavy chain-only antibody of claim 1, which is a human antibody.

3. The heavy chain-only antibody of claim 2, which lacks hallmark camelid related amino acid substitutions and has FR2 substitutions which are not found in human antibodies which comprise heavy and light chain.

4. The heavy chain-only antibody of any one of claims 1 to 3, which has a binding affinity of 10 nM or better.

5. A high affinity, antigen specific soluble VH domain which:

lacks hallmark camelid related amino acid substitutions and has FR2 substitutions which are not found in antibodies which comprise heavy and light chain;

shows increased net hydrophobicity within CDR1 and an increased number of charged amino acids present in CDR3; and

comprises one or more amino acid substitutions within the framework ß-pleated sheet leading to increased hydrophobicity within FR1 and an increased number of charged amino acids present in FR3.

6. The VH domain of claim 5, which is a soluble human VH domain.

7. The soluble VH domain of claim 6, which lacks hallmark camelid-related amino acid substitutions and has FR2 substitutions which are not found in human antibodies which comprise heavy and light chain.

8. The soluble VH domain of any one of claims 5 to 7, which has a binding affinity of 10 nM or better.

9. A V gene segment which, when recombined with a D gene segment and a J gene segment, and following affinity maturation encodes a soluble VH domain according to any one of claims 5 to 8.

10. The V gene segment of claim 9, wherein the sequence of the V gene segment has been engineered by way of at least one substitution, insertion or deletion, or a combination thereof, relative to the natural germ-line sequence of the V gene segment so that it includes codons encoding amino acid residues or amino acid sequences which have been shown to contribute to the solubility and stability of a VH domain.

11. A D gene segment which, when recombined with a V gene segment according to claim 9 or claim 10 and a J gene segment, and following affinity maturation encodes a soluble VH domain according to any one of claims 5 to 8.

12. The D gene segment of claim 11, wherein the sequence of the D gene segment has been engineered by way of at least one substitution, insertion or deletion, or a combination thereof, relative to the natural germ-line sequence of the D gene segment so that it includes codons encoding amino acid residues which have been determined to contribute to maintaining solubility and stability of a soluble VH domain including sequence encoded by the D gene segment.

13. A J gene segment which, when recombined with a V gene segment according to claim 9 or claim 10 and a D gene segment, and following affinity maturation encodes a soluble VH domain according to any one of claims 5 to 8.

14. The J gene segment of claim 13, wherein the sequence of the J gene segment has been engineered by way of at least one substitution, insertion or deletion, or a combination thereof, relative to the natural germ-line sequence of the J gene segment so that it includes codons encoding amino acid residues which have been determined

to contribute to maintaining solubility and stability of a soluble VH domain including sequence encoded by the J gene segment.

15. A heterologous heavy chain-only locus comprising one or more V gene segments according to claim 9 or claim 10, one or more D gene segments according to claim 11 or claim 12, one or more J gene segments according to claim 13 or claim 14 and one or more constant effector region gene segments, each of which encodes an antibody constant effector region lacking CH1 functionality, wherein the gene segments are arranged such that a V, a D and a J gene segment and a constant region gene segment can recombine to produce a rearranged affinity matured heavy chain-only gene locus encoding a high affinity, antigen-specific, soluble heavy chain-only antibody according to any one of claims 1 to 4.

16. The heavy chain-only locus of claim 15, which is arranged to produce a human heavy chain-only antibody or a hybrid antibody comprising a soluble human VH domain and constant region gene segments from another species

17. The heavy chain-only locus of claim 15 or claim 16 wherein the constant region gene segments are of rodent origin, preferably mouse.

18. A transgenic non-human mammal comprising a heterologous heavy chain-only locus according to any one of claims 15 to 17.

19. A transgenic non-human mammal that does not express any functional immunoglobulin light chain genes and comprises a heterologous heavy chain-only locus comprising one or more V gene segments according to claim 9 or claim 10, one or more D gene segments according to claim 11 or claim 12, one or more J gene segments according to claim 13 or claim 14 and one or more constant effector region gene segments, each of which encodes an antibody constant effector region including CH1 functionality, wherein the gene segments are arranged such that a V, a D and a J gene segment and a constant region gene segment can recombine to produce a rearranged affinity matured heavy chain-only gene locus encoding a high affinity, antigen-specific, soluble heavy chain-only antibody.

20. The transgenic non-human mammal of claim 19, wherein the heavy chain-only locus is arranged to produce a human heavy chain-only antibody or a hybrid antibody comprising a soluble human VH domain and constant region gene segments from another species

21. The transgenic non-human mammal of claim 19 or claim 20 wherein the constant region gene segments are of rodent origin, preferably mouse

22. The transgenic non-human mammal of any one of claims 18 to 21, which is a mouse.

23. A method for the production of a high affinity, antigen-specific, soluble heavy chain-only antibody, which comprises challenging a transgenic non-human mammal according to any one of claims 18-22 with an antigen.

24. The method of claim 23, wherein the soluble VH domain of the heavy chain-only antibody is human.

25. A method for obtaining nucleic acid encoding a heavy chain-only antibody chain or soluble VH domain comprising: obtaining long-lived plasma cells or memory B-cells from a transgenic mammal including a heavy chain-only locus, which mammal has been challenged with an antigen; and isolating nucleic acid encoding a heavy chain-only antibody chain or soluble VH domain specific for the antigen from the long-lived plasma cells or memory B-cells.

26. The method of claim 25, wherein the long-lived plasma cells or memory B-cells are purified from the secondary lymph glands, bone marrow or spleen of the antigen-challenged, non-human transgenic mammal.

27. The method of claim 25 or claim 26, wherein the long-lived plasma cells or memory B-cells are purified using cell surface markers on the cells.

28. The method of claim 27, wherein the cell surface marker is the CD138 marker.

29. The method of any one of claims 24 to 26, wherein a population of mRNAs is isolated from the cells and a population of cDNAs encoding heavy chain-only antibody or soluble VH domains is produced from said population of mRNAs.

30. The method of claim 29, wherein:

the population of cDNAs is cloned into an expression vector;

sequences cloned into the cDNA expression vector are optionally transfected into bacteria selected, amplified and colonies picked and recombinant vector DNA purified;

a cell-line capable of expressing, accumulating, secreting or presenting a protein encoded by the expression vector is transformed with the expression vector; and

a cell-line which expresses, accumulates, secretes or presents a heavy chain-only antibody chain which recognises the antigen is selected.

31. The method of any one of claims 25 to 27, wherein a population of mRNAs is isolated from the cells and a population of cDNAs which encode the VH domains of any antibody produced by the cells is produced from said population of mRNAs.

32. The method of claim 31, wherein:

the population of cDNAs which encode the VH domains of any antibody produced by the cells is cloned into an expression vector;

a cell-line capable of expressing, accumulating, secreting or presenting a protein encoded by the expression vector is transformed with the expression vector; and

a cell-line which expresses, accumulates, secretes or presents a VH domain which recognises the antigen is selected.

33. The method of claim 30 or claim 32 wherein the cell-line is a microbial cell-line or a mammalian cell-line.

34. A method of producing a heavy chain-only antibody which binds specifically to an antigen comprising:

(a) immunising a non-human transgenic mammal with the antigen, wherein the mammal expresses heavy chain-only antibodies which lack CH1 functionality in the transcribed and processed heavy chain mRNA;

(b) isolating long-lived plasma cells or memory B-cells from the immunised mammal;

(c) isolating an mRNA population from cells derived from step (b);

(d1) cloning a cDNA population derived from the mRNA of step (c) into an expression vector and expressing the cDNA in a cell-line;

(e1) selecting at least one cell-line which produces a heavy chain-only antibody which binds specifically to the antigen;

or

(d2) cloning a cDNA population comprising cDNAs which encode a VH domains derived from the mRNA of step (c) into expression vector such that a VH fusion protein, which may comprise a heavy chain effector region, is expressed in the cell-line;

(e2) selecting at least one cell line which produces a VH fusion protein which binds specifically to the antigen.

35. The method of any one of claims 25 to 34, wherein the isolated long-lived plasma cells or memory B-cells are immortalised prior to mRNA isolation.

36. The method of any one of claims 25 to 35 wherein any heavy chain-only antibody locus includes at least one dominant selective marker gene.

37. The method of claim 23 or claim 24, which includes the step of immortalising antibody producing cells from the mammal.

38. The method of claim 35 or claim 37, wherein the cells are immortalised by fusion with a myeloma cell.

39. The method of claim 35 or claim 37, wherein the cells are immortalised by transformation with a virus, such as the EBV.

40. The method of any one of claims 25 to 39, further including the step of isolating either the soluble VH domain or a nucleic acid encoding the soluble VH domain from a B-cell or an immortalised cell line producing a heavy chain-only antibody of desired antigen specificity.

41. The method of any one of claims 23 to 40, further including the step of isolating either a soluble VH domain or a nucleic acid encoding a soluble VH domain from a heavy chain-only antibody-producing cell produced by the mammal or from an immortalised heavy chain-only antibody producing cell produced by the mammal by a display approach.

42. Use of a soluble VH domain or a nucleic acid encoding a soluble VH domain obtained by the method of any one of claims 23 to 40 in the production of a heavy chain-only antibody, a VH intrabody, a VH polyprotein, a VH domain complex or a VH fusion.

43. A heavy chain-only antibody, a VH intrabody, a VH polyprotein, a VH domain complex or a VH fusion including at least one VH domain according to any one of claims 5 to 8.

44. A heavy chain-only antibody, a VH intrabody, a VH polyprotein, a VH domain complex or a VH fusion including at least one VH domain according to any one of claims 5 to 8 for use in therapy.

45. Use of a heavy chain-only antibody, a VH intrabody, a VH polyprotein, a VH domain complex or a VH fusion including at least one VH domain according to any one of claims 5 to 8 in the preparation of a medicament.

46. A cassette for producing a VH domain according to any one of claims 5 to 8, which comprises gene sequences encoding FR1, FR2 and FR3, wherein the gene sequences encoding FR1 and FR2 and FR2 and FR3 are separated by sequences allowing the introduction of suitable CDR1- and CDR2-encoding sequences and, at the 3' end of the sequence encoding FR3, there is a sequence allowing the introduction of a sequence encoding the CDR3-encoding sequence.

SOLUBLE "HEAVY-CHAIN ONLY" ANTIBODIES

Field of the Invention

The present invention relates to improved methods for isolating from transgenic non-human mammals a diverse repertoire of functional, soluble, heavy chain-only antibodies and soluble VH domains derived therefrom which are structurally distinct from VH domains derived from antibodies comprising heavy and light chains. Preferably, the heavy chain-only antibodies and soluble VH domains are derived from long-lived plasma cells or memory B-cells.

Background to the Invention

Monoclonal antibodies or variants thereof will represent a high proportion of new medicines launched in the 21st century. Monoclonal antibody therapy is already accepted as a preferred route for the treatment for rheumatoid arthritis and Crohn's disease and there is impressive progress in the treatment of cancer. Antibody-based products are also in development for the treatment of cardiovascular and infectious diseases. Most marketed monoclonal antibody products recognise and bind a single, well-defined epitope on the target ligand (e.g. TNFα).

The structure of antibodies is well known in the art. Most natural antibodies are tetrameric and comprise two heavy chains and two light chains. The heavy chains are joined to each other via disulphide bonds between hinge domains located approximately half way along each heavy chain. A light chain is associated with each heavy chain on the N-terminal side of the hinge domain. Each light chain is normally bound to its respective heavy chain by a disulphide bond close to the hinge domain.

When an antibody molecule is correctly folded, each chain folds into a number of distinct globular domains joined by a more linear polypeptide sequence. For example, the light chain folds into a variable (VL) and a constant (CL) domain. Heavy chains have a single variable domain (VH) adjacent the variable domain (VL) of the light chain, a first constant domain (CH1), a hinge domain and two or three further constant domains. Interaction of the heavy (VH) and light (VL) chain variable domains results in the formation of an antigen-binding region (Fv).

The interaction between the heavy and light chains is mediated by the first constant domain of the heavy chain (CH1) and the constant domain (CL) of the light chain. However, there is also an interface between the heavy chain variable domain

CLAIMS

1. A method for obtaining nucleic acid encoding a heavy-chain only antibody chain comprising:

a) obtaining long-lived plasma cells or memory B-cells from a transgenic mammal including a heavy chain-only locus comprising human VH gene segments, one or more human D gene segments, human J gene segments, and at least one mouse constant region gene segment wherein the human VH gene segments consist of VH1-69, VH4-59, VH3-53, VH3-49, VH4-34, VH3-48, VH3-33, VH3-30, VH3-23, VH1-18, VH3-15, VH4-39, VH1-8, VH3-07, VH2-5, VH4-4, VH1-2, and VH6-1, the human D gene segments comprise at least 20 human D gene segments, the human J gene segments comprise all six human J gene segments, and the mouse constant region gene segment comprises a Cγ lacking CH1, which mammal has been challenged with an antigen; and

b) isolating nucleic acid encoding a heavy chain-only antibody chain specific for the antigen from the long-lived plasma cells or memory B-cells.

2. The method of claim 1, wherein the long-lived plasma cells or memory B-cells are purified from secondary lymph glands, bone marrow or spleen of the antigen-challenged, non-human transgenic mammal.

3. The method of claim 1, wherein the long-lived plasma cells or memory B-cells are purified using cell surface markers on the cells.

4. The method of claim 3, wherein the cell surface marker is the CD138 marker.

5. The method of claim 1, further comprising isolating a population of mRNAs from the cells and producing a population of cDNAs encoding heavy chain-only antibody from said population of mRNAs.

6. The method of claim 5, further comprising:

a) cloning the population of cDNAs into an expression vector;

b) optionally purifying sequences cloned into the cDNA expression vector by transfecting said expression vectors into bacteria and selecting colonies comprising said vectors for amplification;

c) transforming a cell-line capable of expressing, accumulating, secreting or presenting a protein encoded by the expression vector with the expression vector; and

d) selecting a cell-line which expresses, accumulates, secretes or presents a heavy chain-only antibody chain which recognises the antigen.

7. The method of claim 1, further comprising isolating a population of mRNAs from the cells and producing a population of cDNAs which encode the VH domains of any antibody produced by the cells from said population of mRNAs.

8. The method of claim 7, further comprising:

a) cloning the population of cDNAs which encode the VH domains of any antibody produced by the cells into an expression vector;

b) transforming a cell-line capable of expressing, accumulating, secreting or presenting a protein encoded by the expression vector with the expression vector; and

c) selecting a cell-line which expresses, accumulates, secretes or presents a VH domain which recognises the antigen.

9. The method of claim 6 wherein the cell-line is a microbial cell-line or a mammalian cell-line.

10. A method of producing a heavy chain-only antibody comprising a soluble VH and which binds specifically to an antigen comprising:

(a) immunising a non-human transgenic mammal comprising a heavy chain-only antibody locus comprising human VH gene segments, one or more human D gene segments, human J gene segments, and at least one mouse constant region gene segment wherein the human VH gene segments consist of VH1-69, VH4-59, VH3-53, VH3-49, VH4-34,

VH3-48, VH3-33, VH3-30, VH3-23, VH1-18, VH3-15, VH4-39, VH1-8, VH3-07, VH2-5, VH4-4, VH1-2, and VH6-1, the human D gene segments comprise at least 20 human D gene segments, the human J gene segments comprise all six human J gene segments, and the mouse constant region gene segment comprises a Cγ lacking CH1, with the antigen, wherein the mammal expresses heavy chain-only antibodies comprising soluble VHs and which lack CH1 functionality in the transcribed and processed heavy chain mRNA;

(b) isolating long-lived plasma cells or memory B-cells from the immunised mammal;

(c) isolating an mRNA population from cells derived from step (b);

(d) cloning a cDNA population derived from the mRNA of step (c) into an expression vector and expressing the cDNA in a cell-line; and

(e) selecting at least one cell-line which produces a heavy chain-only antibody which binds specifically to the antigen.

11. The method of claim 10, wherein the isolated long-lived plasma cells or memory B-cells are immortalised prior to mRNA isolation.

12. The method of claim 10 wherein any heavy chain-only antibody locus includes at least one dominant selective marker gene.

13. The method of claim 10, which includes the step of immortalising antibody producing cells from the mammal.

14. The method of claim 13, wherein the cells are immortalised by fusion with a myeloma cell.

15. The method of claim 13, wherein the cells are immortalised by transformation with a virus.

16. A method of producing a heavy chain-only antibody comprising a soluble VH and which binds specifically to an antigen comprising:

(a) immunising a non-human transgenic mammal comprising a heavy chain-only antibody locus comprising human VH gene segments, one or more human D gene segments, human J gene segments, and at least one mouse constant region gene segment wherein the human VH gene segments consist of VH1-69, VH4-59, VH3-53, VH3-49, VH4-34, VH3-48, VH3-33, VH3-30, VH3-23, VH1-18, VH3-15, VH4-39, VH1-8, VH3-07, VH2-5, VH4-4, VH1-2, and VH6-1, the human D gene segments comprise at least 20 human D gene segments, the human J gene segments comprise all six human J gene segments, and the mouse constant region gene segment comprises a Cγ lacking CH1, with the antigen, wherein the mammal expresses heavy chain-only antibodies comprising soluble VHs and which lack CH1 functionality in the transcribed and processed heavy chain mRNA;

(b) isolating long-lived plasma cells or memory B-cells from the immunised mammal;

(c) isolating an mRNA population from cells derived from step (b);

(d) cloning a cDNA population comprising cDNAs which encode a VH domains derived from the mRNA of step (c) into expression vector such that a VH fusion protein, which may comprise a heavy chain effector region, is expressed in the cell-line; and

(e) selecting at least one cell line which produces a VH fusion protein which binds specifically to the antigen.

17. The method of claim 16, wherein the isolated long-lived plasma cells or memory B-cells are immortalised prior to mRNA isolation.

18. The method of claim 16, wherein any heavy chain-only antibody locus includes at least one dominant selective marker gene.

19. The method of claim 16, which includes the step of immortalising antibody producing cells from the mammal.

20. The method of claim 19, wherein the cells are immortalised by fusion with a myeloma cell.

21. The method of claim 19, wherein the cells are immortalised by transformation with a virus.

22. A method for obtaining nucleic acid encoding a soluble VH domain comprising:

a) obtaining long-lived plasma cells or memory B-cells from a transgenic mammal including a heavy chain-only locus comprising human VH gene segments, one or more human D gene segments, human J gene segments, and at least one mouse constant region gene segment wherein the human VH gene segments consist of VH1-69, VH4-59, VH3-53, VH3-49, VH4-34, VH3-48, VH3-33, VH3-30, VH3-23, VH1-18, VH3-15, VH4-39, VH1-8, VH3-07, VH2-5, VH4-4, VH1-2, and VH6-1, the human D gene segments comprise at least 20 human D gene segments, the human J gene segments comprise all six human J gene segments, and the mouse constant region gene segment comprises a Cγ lacking CH1, which mammal has been challenged with an antigen; and

b) isolating nucleic acid encoding a soluble VH domain specific for the antigen from the long-lived plasma cells or memory B-cells.

23. The method of claim 22, wherein the long-lived plasma cells or memory B-cells are purified from the secondary lymph glands, bone marrow or spleen of the antigen-challenged, non-human transgenic mammal.

24. The method of claim 22, wherein the long-lived plasma cells or memory B-cells are purified using cell surface markers on the cells.

25. The method of claim 24, wherein the cell surface marker is the CD138 marker.

26. The method of claim 22, further comprising isolating a population of mRNAs from the cells and producing a population of cDNAs encoding heavy chain-only antibody from said population of mRNAs.

27. The method of claim 26, further comprising:

a) cloning the population of cDNAs into an expression vector;

b) optionally purifying sequences cloned into the cDNA expression vector by transfecting said expression vectors into bacteria and selecting colonies comprising said vectors for amplification;

c) transforming a cell-line capable of expressing, accumulating, secreting or presenting a protein encoded by the expression vector with the expression vector; and

d) selecting a cell-line which expresses, accumulates, secretes or presents a heavy chain-only antibody chain which recognises the antigen.

28. The method of claim 22, further comprising isolating a population of mRNAs from the cells and producing a population of cDNAs which encode the VH domains of any antibody produced by the cells from said population of mRNAs.

29. The method of claim 28, further comprising:

a) cloning the population of cDNAs which encode the VH domains of any antibody produced by the cells into an expression vector;

b) transforming a cell-line capable of expressing, accumulating, secreting or presenting a protein encoded by the expression vector with the expression vector; and

c) selecting a cell-line which expresses, accumulates, secretes or presents a VH domain which recognises the antigen.

30. The method of claim 29 wherein the cell-line is a microbial cell-line or a mammalian cell-line.

31. The method of claim 15 or 21, wherein the virus is EBV.