

Claims

1. A method of generating a rat, comprising:
 - (A) injecting into a rat germ cell, rat fertilized oocyte or a rat embryo, a meganuclease specific for Ig gene fragments in heavy and/or light chain loci endogenous to the rat, wherein the meganuclease introduces double-strand breaks into said heavy and/or light chain loci; or (B) injecting into a rat germ cell, rat fertilized oocyte or a rat embryo, an expression vector or nucleic acid encoding a meganuclease specific for Ig gene fragments in heavy and/or light chain loci endogenous to the rat, wherein the meganuclease introduces double-strand breaks into said heavy and/or light chain loci.
2. A rat which is nullizygous for endogenous Ig light chain loci and/or endogenous heavy chain loci.
3. The rat according to claim 2 comprising an artificial immunoglobulin (Ig) locus.
4. The rat according to claim 3, wherein the artificial Ig locus is an Ig light chain locus and/or a Ig heavy chain locus.
5. The rat according to claim 3, wherein the artificial Ig locus comprises an Ig locus comprising fragments of human and rat Ig loci including multiple immunoglobulin gene segments, optionally wherein: (i) at least one variable (V) gene segment is a human V gene segment; and/or (ii) at least one diverse (D) gene segment is a human D gene segment.
6. The rat according to claim 5, wherein the human gene segment comprises: i) naturally occurring sequences of a human Ig gene segment; ii) degenerate forms of naturally occurring sequences of a human Ig gene segment; and/or iii) synthetic sequences encoding a polypeptide sequence that is 85%-95% identical to the polypeptide encoded by a naturally occurring sequence of a human Ig gene segment.
7. The rat according to any one of claims 3 to 6 that is capable of producing immunoglobulins with human idiotypes.
8. A method for producing a monoclonal antibody, comprising: (i) immunizing the rat according to any of claims 3 to 7 with an immunogen; (ii) isolating a monoclonal antibody producing cell from the rat wherein the monoclonal antibody producing cell produces a monoclonal antibody that specifically binds to the immunogen; and (iii) using the monoclonal antibody producing cell to produce the monoclonal antibody that specifically binds to the immunogen, or using the monoclonal antibody producing cell to produce a hybridoma cell that produces the monoclonal antibody and using the hybridoma cell to produce the monoclonal antibody.
9. A method for producing a monoclonal antibody comprising: (i) immunizing the rat according to any of claims 3 to 7 with an immunogen; (ii) isolating a monoclonal antibody producing cell from the rat wherein the monoclonal antibody producing cell produces a monoclonal antibody that specifically binds to the immunogen; (iii) isolating from the monoclonal antibody producing cell

a monoclonal antibody nucleic acid which encodes the monoclonal antibody that specifically binds to the immunogen; and (iv) using the monoclonal antibody nucleic acid to produce the monoclonal antibody that specifically binds to the immunogen, wherein optionally the monoclonal antibody has a human idiotype.

10. A method for producing a monoclonal antibody comprising: (i) immunizing the rat according to any of claims 3 to 7 with an immunogen; (ii) isolating a monoclonal antibody producing cell from the rat wherein the monoclonal antibody producing cell produces a monoclonal antibody that specifically binds to the immunogen; (iii) isolating from the monoclonal antibody producing cell a monoclonal antibody nucleic acid which encodes the monoclonal antibody that specifically binds to the immunogen; (iv) modifying the monoclonal antibody nucleic acid to produce a recombinant nucleic acid encoding a fully human monoclonal antibody; and (v) using the recombinant nucleic acid encoding a fully human monoclonal antibody to produce the encoded fully human monoclonal antibody.
11. The method according to any one of claims 8 to 10, wherein the method further comprises the steps of: i) purifying the antibody; and ii) admixing the antibody with an appropriate pharmaceutical carrier suitable for administration to patients.
12. A cell obtainable by the method of claim 8.
13. A transfected cell comprising a nucleic acid encoding the monoclonal antibody defined in claim 9.
14. The transfected cell of claim 13 producing a fully human monoclonal antibody.
15. An isolated nucleic acid encoding the monoclonal antibody obtainable by the method of claim 9, wherein the artificial locus comprises at least one human J gene segment.
16. An immunoglobulin comprising a polypeptide sequence encoded by the nucleic acid of claim 15.
17. The immunoglobulin of claim 16, wherein the immunoglobulin has a constant (C) region which is selected from (i) a rat constant C region, or (ii) an artificial constant (C) region, wherein the artificial constant region is derived from human and rat constant regions, optionally wherein the artificial (C) region has a human IgG CH1 domain and a rat IgG CH2 and CH3 domain.
18. A transfected cell comprising the nucleic acid of claim 15.
19. The transfected cell of claim 18 producing a fully human monoclonal antibody.

Claims

1. A method of generating a rat, comprising:

(A) injecting into a rat germ cell, rat fertilized oocyte or a rat embryo, a meganuclease specific for Ig gene fragments in heavy and/or light chain loci endogenous to the rat, wherein the meganuclease introduces doublestrand_breaks into said heavy and/or light chain loci; or (B) injecting into a rat germ cell, rat fertilized oocyte or a rat embryo, an expression vector or nucleic acid encoding a meganuclease specific for Ig gene fragments in heavy and/or light chain loci endogenous to the rat, wherein the meganuclease introduces double-strand breaks into said heavy and/or light chain loci.

2. A rat which is nullizygous for endogenous Ig light chain loci and/or endogenous heavy chain loci.

3. The rat according to claim 2 comprising an artificial immunoglobulin (Ig) locus.

4. The rat according to claim 3, wherein the artificial Ig locus is an Ig light chain locus and/or a Ig heavy chain locus.

5. The rat according to claim 3, wherein the artificial Ig locus comprises an Ig locus comprising fragments of human and rat Ig loci including multiple immunoglobulin gene segments, optionally wherein: (i) at least one variable (V) gene segment is a human V gene segment; and/or ii) at least one diverse (D) gene segment is a human D gene segment.

6. The rat according to claim 5, wherein the human gene segment comprises: i) naturally occurring sequences of a human Ig gene segment; ii) degenerate forms of naturally occurring sequences of a human Ig gene segment; and/or iii) synthetic sequences encoding a polypeptide sequence that is 85%-95% identical to the polypeptide encoded by a naturally occurring sequence of a human Ig gene segment.

7. The rat according to anyone of claims 3 to 6 that is capable of producing immunoglobulins with human idiotypes.

8. A method for producing a monoclonal antibody, comprising: (i) immunizing the rat according to any of claims 3 to 7 with an immunogen; (ii) isolating a monoclonal antibody producing cell from the rat wherein the monoclonal antibody producing cell produces a monoclonal antibody that specifically binds to the immunogen; and (iii) using the monoclonal antibody producing cell to produce the monoclonal antibody that specifically binds to the immunogen, or using the monoclonal antibody producing cell to produce a hybridoma cell that produces the monoclonal antibody and using the hybridoma cell to produce the monoclonal antibody.

9. A method for producing a monoclonal antibody comprising: (i) immunizing the rat according to any of claims 3 to 7 with an immunogen; (ii) isolating a monoclonal antibody producing cell from the rat wherein the monoclonal antibody producing cell produces a monoclonal antibody that specifically binds to the immunogen; (iii) isolating from the monoclonal antibody producing cell a monoclonal antibody nucleic acid which encodes the monoclonal antibody that specifically binds to the immunogen; and (iv) using the monoclonal antibody nucleic acid to produce the monoclonal antibody that specifically binds to the immunogen, wherein optionally the monoclonal antibody has a human idotype.

10. A method for producing a monoclonal antibody comprising: (i) immunizing the rat according to any of claims 3 to 7 with an immunogen; (ii) isolating a monoclonal antibody producing cell from the rat wherein the monoclonal antibody producing cell produces a monoclonal antibody that specifically binds to the immunogen; (iii) isolating from the monoclonal antibody producing cell a monoclonal antibody nucleic acid which encodes the monoclonal antibody that specifically binds to the immunogen; (iv) modifying the monoclonal antibody nucleic acid to produce a recombinant nucleic acid encoding a fully human monoclonal antibody; and (v) using the recombinant nucleic acid encoding a fully human monoclonal antibody to produce the encoded fully human monoclonal antibody.

11. The method according to anyone of claims 8 to 10, wherein the method further comprises the steps of: i) purifying the antibody; and ii) admixing the antibody with an appropriate pharmaceutical carrier suitable for administration to patients.
12. A cell obtainable by the method of claim 8.
13. A transfected cell comprising a nucleic acid encoding the monoclonal antibody defined in claim 9.
14. The transfected cell comprising the monoclonal antibody nucleic acid which encodes the monoclonal antibody that specifically binds to the immunogen as defined in claim 10.
15. An isolated nucleic acid encoding the monoclonal antibody obtainable by the method of claim 9, wherein the artificial locus comprises at least one human J gene segment, wherein the monoclonal antibody is a chimeric immunoglobulin which comprises a portion of human immunoglobulin polypeptide sequence and a portion of rat immunoglobulin polypeptide sequence.
16. A chimeric immunoglobulin comprising a polypeptide sequence encoded by the nucleic acid of claim 15, wherein the chimeric immunoglobulin comprises a portion of human immunoglobulin polypeptide sequence and a portion of rat immunoglobulin polypeptide sequence.
17. The immunoglobulin of claim 16, wherein the immunoglobulin has a constant (C) region which is selected from (i) a rat constant C region, or (ii) an artificial constant (C) region, wherein the artificial constant region is derived from human and rat constant regions, optionally wherein the artificial (C) region has a human IgG CH1 domain and a rat IgG CH2 and CH3 domain
18. A transfected cell comprising the nucleic acid of claim 15.